LIFE HISTORY, MORPHOLOGY AND HOST CHOICE AMONG POPULATIONS OF *Blissus leucopterus hirtus* MONTANDON (HEMIPTERA: BLISSIDAE) IN QUEBEC AND ATLANTIC CANADA

ROBYN AULD

Life history, Morphology and Host Choice among Populations of *Blissus leucopterus hirtus* Montandon (Hemiptera: Blissidae) in Quebec and Atlantic Canada

DOCTORAL THESIS

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Department of Biology, Faculty of Science
Memorial University of Newfoundland

Abstract

Blissus leucopterus hirtus Montandon (Hemiptera: Blissidae) (hairy chinch bug), is a pest of turfgrass in Quebec and Atlantic Canada that causes considerable damage to turf and lawns. This research investigated the influence of environmental heterogeneity on the phenology, morphology and host preference of *B. leucopterus hirtus*. Populations of *B. leucopterus hirtus* in St. John's, NL displayed the lowest threshold for egg development and the highest rate of egg development compared with other regions across the insect's range, indicating environmental influence attributable to shorter and cooler summers in the St. John's region. This adaptation was also reflected in a more compact phenology compared to other regions. Cumulative Degree Days and Julian Days were both effective in predicting the appearance of 2nd instars, a critical milestone for pest managers in conducting appropriately timed pesticide application.

There was no difference in tertiary sex ratio among sites at a local scale, but there was an greater number of males than females in Fall 2004 and 2005 compared to the sprang, suggesting differential mortality of dispersal. Wing form ratios varied at the local scale and over time, with an increase in brachypters over two years indicating increasingly established populations. Differences in habitat at the local and regional scale were sufficient to produce differences in insect size, with the smallest insect size rankings in St. John's, NL, where egg development rates were also most rapid.

Differences in host choice among 1st instars of *B. leucopterus hirtus* were observed at the local and regional level, demonstrating flexibility in host choice among

populations. Preference was affected by age of insect, with 1st instars favouring Kentucky bluegrass, while 5th instars were less discriminant. Tendency for aggregation in later instars appeared to influence choice. Based on these results, pest managers should be better able to predict host vegetation based on insect phenology and potentially apply alternative methods of control such a vacuuming when populations are likely to be found on specific host plants as a result of either feeding preference or other behaviour tendencies such as aggregation.

The results of this thesis confirm the influence of environmental heterogeneity on the phenology, morphology and host preference of *B. leucopterus hirtus* and the subsequent need for regional specificity in pest management, along with consideration of insect age and populations dynamics in host choice research.

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List of Abbreviations

AAFC: Agriculture and Agri-Food Canada

ANOVA: Analysis of Variance

BLR: Binary Logistic Regression

CDD: Cumulative Degree Days

IPM: Integrated Pest Management

JD: Julian Day

NB: New Brunswick

NL: Newfoundland and Labrador

NS: Nova Scotia

PQ: Province de Québec

Co-authorship Statement

In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of co-authors was primarily through the provision of guidance and consultation on general approach, experimental design, data analysis and manuscript editing.

I hereby declare that this thesis incorporates material that is the result of joint research, as follows:

i) The initial idea to investigate the life history of *Blissus leucopterus hirtus* was in response to industry interest and research dollars provided by Landscape Newfoundland and Labrador. This idea was proposed to the author as a prospective project by Dr. Murray Colbo and the design of that project was developed in consultation with Dr. Murray Colbo and Dr. Peggy Dixon.

ii) The majority of the practical aspects of this project (including insect and data collection) were completed by the author. Technical assistance was provided at times by other students and Janet Coombes, the technician of Dr. Peggy Dixon.

iii) The data analysis was completed entirely by the author, with guidance from Dr. Keith Lewis, Dr. Ken McRae and Dr. David Schneider. Consultation from Dr. Murray Colbo and Dr. Peggy Dixon was also incorporated into the statistical approach.

iv) The manuscript was prepared by the author with comments and editing provided by Dr. Murray Colbo, Dr. Peggy Dixon and Dr. Paul Marino.

I am aware of the Memorial University of Newfoundland and Labrador Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis.

I certify that, with the above qualifications, this thesis, and the research to which it refers, is the product of my own work.

Chapter 1: Introduction

1.1 Context

Implicit in a strategy to protect the natural environment, there is a global movement to reduce reliance on chemical pesticides. This approach involves reducing both the amount and frequency of pesticide application while still maintaining acceptable control of pest species. Known as 'Integrated Pest Management' (IPM), this approach requires a thorough understanding of the ecological facets of insect population strategy (Norris et al. 2002) so that pest managers can interpret natural features of insect phenology, morphology and behaviour, and respond with specifically designed control measures at appropriate times. One insect of interest, *Blissus leucopterus hirtus* Montandon (Hemiptera: Blissidae) (hairy chinch bug) (Fig. 1.1), is a widespread turfgrass pest in Quebec and Atlantic Canada that causes considerable damage to turf and lawns, an industry worth approximately 5 billion dollars a year in Canada (AAFC 2005). Effective management of this pest is aided by knowledge of its ecology, with geographical specificity in life strategy serving as a cornerstone of integrated control measures. This research therefore focuses on providing ecologically based information on key life strategy indicators among local and regional populations to validate the need for population specific management practices.

FIGURE 1.1. Adult brachypterous *Blissus leucopterus hirtus*. Copyright © 2008 Tom Murray. Used with permission. http://www.bugguide.net.

This thesis began as a master's project, supported by a local landscape association, with the goal of determining the number of annual cohorts of *B. leucopterus hirtus* occurring in St. John's, Newfoundland and Labrador (NL) and the environmental factors determining success of populations at various locations within the city. Through this personal observation during this initial research, it appeared that populations of this insect separated by fewer than 5 km displayed differences in life history, including wing form ratio and timing of naturally occurring events in the insect's life cycle. This immediately raised important questions regarding the validity of 'one size fits all' management practices that were being applied in St. John's, which were based on

phenological research conducted in provinces and states outside of Newfoundland and Labrador. For example, guides for this insect suggest pesticide application when the majority of *B. leucopterus hirtus* nymphs are in the 2nd and 3rd instar (Emmons 2000, Tashiro 1987) but the calendar date for the occurrence of these key instars in St. John's was found to differ from those in other parts of the insect's distribution, and even between sites within the city. In New Brunswick (NB) local variation in phenology among locations within that province was also observed (Wellwood et al. 2002). It has become clear that further characterization of these discrepancies is necessary to improve ecologically based management, particularly the timing and efficacy of pesticide application. Despite this, for practical purposes, local pest management companies and other pesticide applicators routinely refer to general calendar dates when planning spray periods. A locally centered, more environmentally friendly management strategy requires an understanding of the life history variation among and within populations at the local and regional scale.

1.2 Theoretical Foundation

Searching for an ecological model, on which to base a research program designed to elucidate these phenological inconsistencies, led to T. R. E. Southwood's 'habitat as a templet' model, which he proposed in his seminal address to the British Entomological Society in 1977 (Southwood 1977). Although Christer Solbreck presented similar ideas at a symposium entitled 'Evolution of escape in space and time' held at the XV International Congress of Entomology in Washington, DC in August, 1976, the contents of this symposium were not published until two years later (Solbreck 1978). Southwood was the

first to publish these ideas and, therefore, has received the majority of credit. According to Southwood's habitat templet model, an individual 'chooses' to engage in behaviour 'here and now', 'here and later', 'elsewhere and now' or 'elsewhere and later' in response to the environmental signals it receives from the habitat in which it finds itself. These choices are molded through selection of strategies that give optimal reproductive success. Thus, success in achieving optimal levels of feeding, shelter and survival of offspring associated with each option should be affected by the favourability of conditions, the probability of being in a suitable habitat (either 'now' or 'later' and/or 'here' or 'elsewhere') and the level of uncertainty regarding these favourable environmental factors over time. This is especially relevant in heterogeneous environments and in areas of seasonality where uncertainty is a dominant factor. Since Southwood first published the habitat templet model, hundreds of papers have cited this unifying theory connecting the evolution of life history strategies to habitat characteristics (Statzner et al. 2001).

The ecological implications of Southwood's habitat templet model are that insects invest resources according to the most favourable options, based on environmental cues they receive. For example, if environmental signals alert the insect to a short or unpredictable period of optimal conditions for growth, growth rates may be accelerated to ensure completion of development (Danks 2006, Danks 2007). The effects of these life strategy 'decisions' also extend beyond phenology. For instance, the wing morph of an organism is the consequence of a series of trade-offs in reproductive potential, which include both dispersal and fecundity, a topic that has been extensively explored and reviewed elsewhere (Denno et al. 1989, Roff 1994, Zera and Mole 1994, Zera and Denno

1997, Langellotto et al. 2000, Langellotto and Denno 2001, Crnokrak and Roff 2002, Danks 2006, Danks 2007). If, during development, environmental signals indicate that conditions 'here and now' are unsuitable for reproduction, hormonal cues in dimorphic insects may influence gene expression and promote development of a macropterous form for dispersal (Harrison 1980). Descriptions in the literature on *B. leucopterus hirtus* often refer to a single population's dimorphic wing ratio (Leonard 1966, Vittum et al. 1999) but preliminary observations in St. John's revealed there was no consistent ratio among local populations, suggesting diverse ecological influences. Because *B. leucopterus hirtus* has a dimorphic wing form, the effect of environment on multiple geographic scales on the fitness and wing form of this insect is of interest within the context of Southwood's model, and may provide further insight into the environmental determinants driving wing form in this species.

The general literature on ecological adaption in insects reveals the potential for environmental influence on multiple life strategy indicators including phenology, morphometrics, wing form and host preference, characteristics for which variation has important implications for developing effective control strategies. Thus, the current use of generalities in *B. leucopterus hirtus* life history to develop control strategies across regions is inaccurate, and may lead to poor management strategies that could be ineffective and/or lead to overuse of pesticides when the target instar is absent. Furthermore, previous research on host preference in *B. leucopterus hirtus* shows little evidence of development stage being taken into account in a variety of host choice experiments, despite reference in the literature to shifts in host preference throughout

ontogeny in other species (Stockhoff 1993), and in taxa closely related to *B. leucopterus hirtus* (Headlee and Walker 1913). Thus, it became clear that Southwood's habitat templet model would serve as an appropriate framework in which to place the variable life strategy of *B. leucopterus hirtus* into an ecological and applied context. This thesis does not aim to test Southwood's model, but rather to use this well supported ecological hypothesis as a framework in which to evaluate patterns and variation observed among populations of *B. leucopterus hirtus.*

1.3 Current Need and Opportunity for Research

There is a striking lack of ecological knowledge on the variation in phenology, morphometrics and host choice among populations of *B. leucopterus hirtus*. There has been considerable research on turfgrass tolerance to, and pesticide effectiveness on this insect (Baker et al. 1981, Mathias et al. 1990, Richmond and Shetlar 2000, Yue et al. 2000) and related subspecies (Mize et al. 1980, Ahmad et al. 1984, Negrón and Riley 1985, Mize and Wilde 1986, Meehan and Wilde 1989, Wilde 1997), yet there has been little effort to synthesize these studies into a more theoretically sound, ecologically founded management approach.

An assessment of the variation among populations of *B. leucopterus hirtus* at multiple geographic scales is a necessary first step in characterizing ecological variation among populations of this insect so that management can be both effective and environmentally sound. Moreover, consideration of the potential for plasticity in life history traits is critical in summarizing the factors potentially responsible for the success

of populations so that potential mitigation may be achieved. Lastly, insights into the ecology of *B. leucopterus hirtus* may allow researchers to better predict damage and potential range expansion for this insect that may occur as a result of adaptation, plasticity and/or newly available habitats as a consequence of climate change. Evidence of this possibility was a recently published in a study which showed an increase in insect multivoltism in northern European moth communities between 1993-2006, in correlation with warmer temperatures (Pöyry et al. 2011). Should *B. leucopterus hirtus* be shown to possess similar capacity to adapt quickly to both novel and changing habitats, range expansion can be expected.

The range of climatic conditions across the Canadian distribution of *B. leucopterus hirtus* provides an opportunity to explore the effect of climate variation on the life strategy of this insect. In particular, questions arise as to whether the increase in damage caused by this pest in St. John's in the past decade results from adaptations to this environment, and what population characteristics might explain how it has managed to respond positively under the environmental conditions present there. The St. John's area of Newfoundland is characterized by milder winters and relatively cooler summers compared to other areas in the insect's range (Banfield 1983) and provides a distinct environmental templet in comparison to other geographical areas of the insect's range. By focusing on a large cross section of the insect's Canadian range, and comparing life strategy of populations of *B. leucopterus hirtus* at both a local (St. John's, NL) and regional (Quebec and Atlantic Canada) scale, it should become clearer to what degree variation in life strategy is present. Exploring the extent to which *B. leucopterus hirtus*

conforms to Southwood's model of habitats serving as life history templets should lead to a better understanding of the evolutionary selection of traits that have made this insect so successful.

The present study focused on three life history traits: phenology, morphology and host choice, in the Quebec and Atlantic Canadian range of *B. leucopterus hirtus*, with a more detailed local analysis of populations in St. John's, Newfoundland. Using Southwood's habitat templet model as a framework for contextualizing the relationships between this insect and its environment at a local and regional level, this research investigated the influence of environmental heterogeneity on the phenology, morphology and host preference of *B. leucopterus hirtus* for the purposes of better informing current ecologically based management strategies. These strategies rely on phenology, as well as indicators of fitness and behaviour, to manage this pest. By identifying variation among key indicators of life strategy, turf managers should now be better able to assess population fitness, conduct appropriately timed pesticide application, predict pest migration and plant suitable host vegetation to mitigate pest damage. In the process of identifying this variation, this research should make a valuable contribution to the field of ecological entomology by confirming the need for more regionally based IPM, particularly at northern latitudes, which are potentially subject to dramatic change in response to climate change.

1.4 Description of *Blissus leucopterus hirtus*

1.4.1 Taxonomy and Distribution

A.E. Montandon first described *Blissus hirtus*, as it was then named, in 1893 from a single specimen collected in Hazelton, Pennsylvania (Montandon 1893). However, earlier records of specimens collected and identified as *Blissus leucopterus* from New York (Fitch 1856, Lintner 1883, van Duzee 1886) and Massachusetts (Fitch 1856) were most likely *Blissus leucopterus hirtus* (Leonard 1966). In 1918, Barber relegated *Blissus hirtus* to a variety [sic] of the *leucopterus* complex (Barber 1918), which is now properly known as a subspecies.

Based on his analysis of morphological characteristics and crossing experiments, Leonard (1966) confirmed *hirtus* as a subspecies of the *leucopterus* complex. He identified only small morphological differences between *B. leucopterus leucopterus* and *B. leucopterus hirtus* and found evidence of interbreeding. However, he considered discrepancy in ease of rearing and potential host differences to support separation into subspecies and suggested that these two subspecies are most likely parapatric in their distribution.

The following is an excerpt from Leonard (1968) and consists of a key for differentiating between species of *Blissus* and subspecies of *Blissus leucopterus*.

8. General coloration gray; pronotum pruinose, concolorous gray, except distal margin.....9
General coloration piceous, with some gray-pruinosity on head and anterior lobe of pronotum; posterior lobe, scutellum, and abdomen not gray.....10

9. Length of abdomen and scutellum less than 3 times width of abdominal tergite V.....*arenarius arenarius*
Length of abdomen and scutellum more than 3 times width of abdominal tergite V.....*arenarius maritimus*

10. Abdominal sternum piceous with setae straw-yellow or yellow .....*leucopterus hirtus*
Abdominal sternum castaneous with setae silver or light straw-yellow.....*leucopterus leucopterus*

The general taxonomy of *Blissus leucopterus hirtus* has changed little since 1966, with the exception of a higher level revision. In his re-evaluation of the infraorder Pentatomomorpha (Hemiptera: Heteroptera), Henry (1997) determined the group to be paraphyletic and created 11 new families including Blissidae, into which *B. leucopterus hirtus* was placed. *Blissus leucopterus hirtus* is considered one of several species/subspecies making up the *leucopterus* complex and one of the three or four (depending on the author) species/subspecies of economic importance (Baker et al. 1981, Vittum et al. 1999, Anderson et al. 2006).

The current taxonomic classification is as follows:

Class ***Insecta***

Order ***Hemiptera***

Suborder ***Heteroptera***

Superfamily ***Lygaeoidea***

Family ***Blissidae***

Genus **Blissus**

Species **leucopterus**

Subspecies **hirtus**

Considered an insect native to North America (Henry and Froeschner 1988), the current Canadian range of *B. leucopterus hirtus* extends from Ontario eastward to Newfoundland. Within the United States, the range extends as far west as eastern Minnesota and south into North Virginia. The geographical range of *Blissus leucopterus hirtus* partially overlaps the range of *Blissus leucopterus leucopterus*, but the latter is considered to be primarily a pest of small grains, sorghum and corn (Negrón and Riley 1990, Vittum et al. 1999), while *B. leucopterus hirtus* is considered a turfgrass pest. For the purpose of this study, turfgrass refers to those varieties and cultivars of grass traditionally used on residential lawns and at outdoor sport and game venues.

1.4.2 Damage

Damage by *B. leucopterus hirtus* is most often associated with dense aggregations of feeding nymphs that create patches of dead grass in lawns and other green areas.

Further detail concerning feeding mechanisms and physiology is provided by Anderson et al. (2006). During periods of high activity, the insect is commonly found in large numbers around the perimeter of damaged patches. In areas of very high infestation, entire lawns may be visibly damaged. Feeding is selective on certain grasses, and many broadleaf species are often unharmed by the insect (Fig. 1.2). Damage is most often apparent in the late summer during periods of high nymphal activity and when there is little rainfall. Visible damage appears to be exacerbated by hot, dry weather. While this may be due to combined stress caused by feeding and lack of water, specific causes of increased visible damage have not been identified.

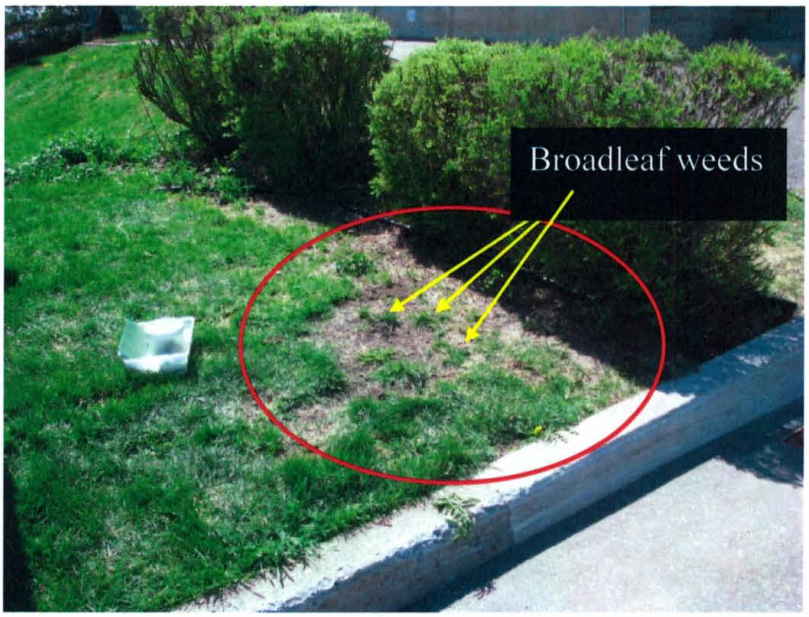

FIG 1.2. Area of *B. leucopterus hirtus* infestation and damage on a residential lawn in Quebec City, PQ. Photograph by Robyn Auld (2008).

1.4.3 Enemies and Natural Control

Natural enemies of *B. leucopterus hirtus* include members of the genus *Geocoris* Say (Hemiptera: Lygaeidae) (big eyed bugs) (Fig. 1.3) and at least seven additional arthropod predators, including at least one mite (Vittum et al. 1999). Another commonly observed cause of death in *B. leucopterus hirtus* is infection by *Beauveria bassiana* (Bals.-Criv) Vuill (Hypocreales: Cordycipitaceae), an entomophagous fungus. Mortality due to this fungus is increased under warm, wet conditions (Headlee and Walker 1913). Parasitism by *Eumicrosoma benefica* Gahan (Hymenoptera: Scelionidae) can also cause mortality in *B. leucopterus hirtus* (Mailloux and Streu 1981).

Important agents used in attempts to control *B. leucopterus hirtus* are species of endophytic fungi (ex. *Neotyphodium coenophialum* (Morgan-Jones & W. Gams) Glenn, C.W. Bacon & Hanlin (Hypocreales Clavicipitaceae)) found naturally and inoculated into turfgrass cultivars. Certain cultivars, including fescues and ryegrasses (but not Kentucky bluegrasses) may be "enhanced" with fungi that cause toxicity to *B. leucopterus hirtus*. At least two studies show individuals of *B. leucopterus hirtus* are able to distinguish between endophyte infected and non-infected grasses with most cases resulting in host avoidance of infected tillers (Carrière et al. 1998, Bourassa et al. 2007).

FIGURE 1.3. *Geocoris bullatus* feeds on a nymph of *B. leucopterus hirtus*. Photograph by Robyn Auld (2006).

1.5 Life History Parameters of *Blissus leucopterus hirtus*

1.5.1 Phenology

The seasonal life cycle of *B. leucopterus hirtus* commences with overwintered adults emerging from sheltered location, including turf tufts and even overturned tables, in the spring; these are called 'spring adults'. The temperature at which spring adults become active is estimated, based on a population in New Jersey, to be 7°C (Mailloux and Streu 1981). Spring adults mate and produce eggs. The fecundity of females reported under field conditions varies tremendously from a mean of 6.87 eggs per female (Mailloux and Streu 1981) to estimates exceeding 400 eggs per female (Potter 1998). These eggs, which measure less than 1 mm in length (Vittum et al. 1999), are typically laid in clusters in small crevices within grass and other ground vegetation. In the days prior to hatching, the eggs turn a deep orange red and the embryo becomes visible

through the chorion (Luginbill 1922). *Blissus leucopterus hirtus* is hemi-metabolous and has 5 nymphal instars. Nymphal life stages are distinguishable by both morphological characteristics (body colour and wing pad development) and head capsule size (Mailloux and Streu 1981, Fig. 1.4). Adult *B. leucopterus hirtus* are approximately 3-4 mm in length and are dimorphic, exhibiting both brachypterous (stunted, short) and macropterous (large) wing forms. Previous research at sites across the insect's geographic range has shown populations may be univoltine or bivoltine (Potter 1998). Throughout the insect's range, some oviposition, hatching and early instar nymphs often occur late in the fall but these nymphs do not reach the adult stage before winter. The lack of nymphs in spring suggests they are unlikely to survive through the winter (Leonard 1966, Wellwood et al. 2002).

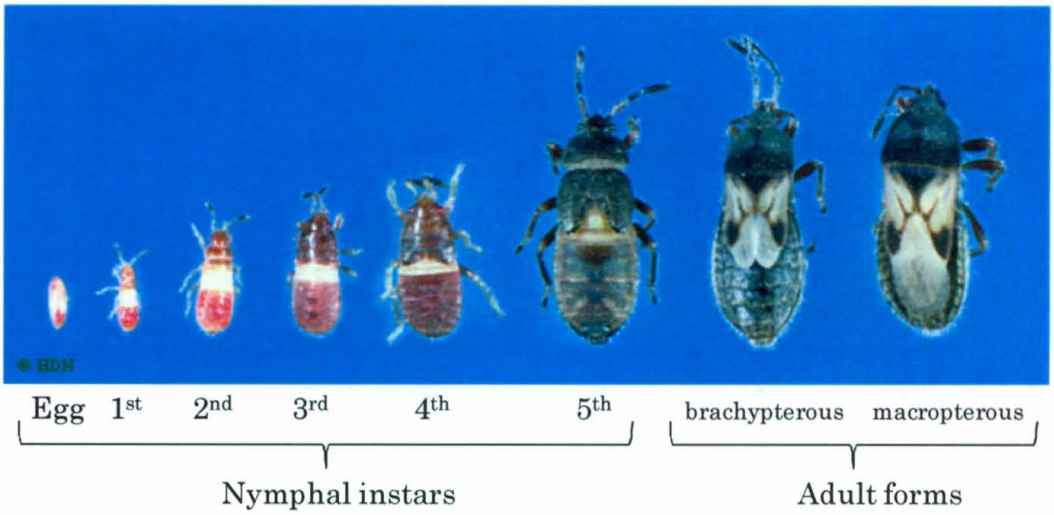

FIGURE 1.4. Life stages *of Blissus leucopterus hirtus*, including both the brachypterous and macropterous adult forms. Adults are approximately 3-4 mm in length. Photograph modified from H. Niemczyk. Original internet source unknown. Used with permission.

There have been few studies that have attempted to document the complete life cycle of *B. leucopterus hirtus* (Mailloux and Streu 1981, Wellwood et al. 2002). Supplemental information comes from partial studies of this insect's life cycle (Liu and McEwen 1979, Sears et al. 1980). Taken together, there is strong evidence that phenological variation exists among regional localities. Bivoltine populations of *B. leucopterus hirtus* have been reported from New Jersey (Mailloux and Streu 1981), in Long Island, New York (Maxwell and MacLeod 1936), Connecticut (Johnson 1941), and Ohio (Polivka 1963). In Ontario and New Brunswick, populations were described as univoltine (Liu and McEwen 1979, Wellwood et al. 2002) although in New Brunswick a second cohort was reported to have initiated but did not complete development. At the commencement of this study it was not known how the phenology of *B. leucopterus hirtus* in St. John's compared to other regions, which was a main driver for the initial research in this thesis. The provincial pest management association recognized this as a key piece of information required to adopt management strategies specific to the city, and eventually the province.

1.5.2 Adult Morphology

Adult size ranges between 3.0 mm and 4.0 mm in length and approximately 1 mm in width (Vittum et al. 1999). In general, females tend to be larger than males. The sexes are easily distinguishable by characteristics on the ventral abdomen: the male has a rounded abdomen with a bulbous terminal segment containing the male genitalia, while the female's abdomen has a medial ridge with a v-shaped protruding ovipositor. *Blissus leucopterus hirtus* exhibits dimorphic wing form, which means that adults display either

macropterous wings (large/developed) or brachypterous wings (small/underdeveloped). Wing form can serve as an important indicator of population dynamics; the degree of macroptery in an insect population may be directly related to the permanence of the habitat (Southwood 1962). The expression of wing morph in insects represents a complex interplay between both environmental and genetic influences, because change can be induced through either environmental manipulation or artificial selection (Zera and Tiebel 1988, Fujisaki 1993, West-Eberhard 2003). Little is understood about the factors that influence wing morphology in *B. leucopterus hirtus*. Leonard (1966) recorded the percentage of brachypters in a single population of 538 individuals at 63.7%. Mailloux and Streu (1981) recorded a maximum percentage occurrence of macroptery at 50% in New Jersey. The latter authors also noted that, in many populations, more females than males were macropterous (Mailloux and Streu 1981). However, they also noted a declining proportion of macropters with time in single cohorts over the course of one summer, suggesting dispersal or differential mortality between the sexes and/or wing forms had occurred. Further investigation is required to determine the true relation between environmental factors and wing form in *B. leucopterus hirtus*.

1.5.3 Host Choice

B. leucopterus hirtus is a phytophagous insect that uses piercing and sucking mouthparts to feed on liquid nutrients from the crowns and stems of primarily cool season turfgrasses including red fescue, perennial ryegrass, bentgrass and Kentucky bluegrass (Vittum et al. 1999). Movement between hosts is common, and the closely related *Blissus leucopterus leucopterus* has been observed shifting between small grains

and corn hosts throughout the year (Headlee and Walker 1913, Packard 1937, Lamp and Holtzer 1980). This behavior may be partially influenced by nutritional quality and availability of hosts as observed in *Blissus occiduus*, which shifts host vegetation throughout the year depending on these factors (Eickhoff et al. 2004). In addition to nutritional quality, architecture of plants has been suggested as a possible determinant of host choice in *Blissus occiduus* (Eickhoff et al. 2006).

The ontogeny of *B. leucopterus hirtus* may also influence optimal host preference because of the insect's tendency to aggregate at later instars. The degree of aggregation in *B. leucopterus hirtus* increases throughout its life history (Mailloux and Streu 1982) and towards later instars, high densities of *B. leucopterus hirtus* are often observed aggregating in grass tufts (e.g. fescues) more so than in other grass species or cultivars in close proximity (Slater 1976, Eickhoff et al. 2004). This suggests that as either internal (physiological) or external (environmental) conditions change, the benefits of aggregation increase and/or certain cultivars offer nutritional or architectural benefits over previously occupied grasses. Movement to more protective grasses in anticipation of less favourable conditions ('elsewhere and later') is a classic example of habitat shaping life history strategy, as proposed by Southwood (1977).

Although there has been a considerable volume of research on the host preference and associated plant resistance in related Blissidae (Stuart et al. 1985, Meehan and Wilde 1989) there has been little research on the factors influencing host preferences of *B. leucopterus hirtus*. There is a need for improvement of pest management and thus to characterize the variability of host choice in relation to development stage and local and

regional geographic patterns as suggested by Southwood's model so that managers can predict on what food source they will most likely find the majority of a population at a given point in its phenology. This is a important step for placing previous and future studies within a geographical context, and from this, into a more ecologically sound plan for managing this pest.

1.6 *Blissus leucopterus hirtus* on the Island of Newfoundland and across its Canadian range

1.6.1 Climate

The geographic range of *B. leucopterus hirtus* populations from Quebec and Atlantic Canada provides a spectrum of environmental conditions, including differences in temperature, rainfall and total precipitation with which to compare life history parameters. The presence of *B. leucopterus hirtus* in Newfoundland, the only subspecies of the *leucopterus* complex found on the island of Newfoundland, presents a valuable opportunity to study habitat-induced population variation, using Southwood's habitat templet model as a theoretical foundation. In St. John's, Newfoundland, the climate is characterized by milder winters and relatively cooler summers compared to more inland areas such as Quebec City, PQ and Fredericton, NB (Banfield 1983) (Fig. 1.5). Compared with the other locations examined in this study, St. John's also has the highest annual precipitation. In addition, Newfoundland is an island and therefore geographically isolated. While there is transport to and from the island, it is highly probable that inter-population dispersal between island and mainland populations is lower than among populations on the Canadian mainland. It is uncertain what, if any, life strategy

adaptations have been made by this insect to succeed in these conditions.

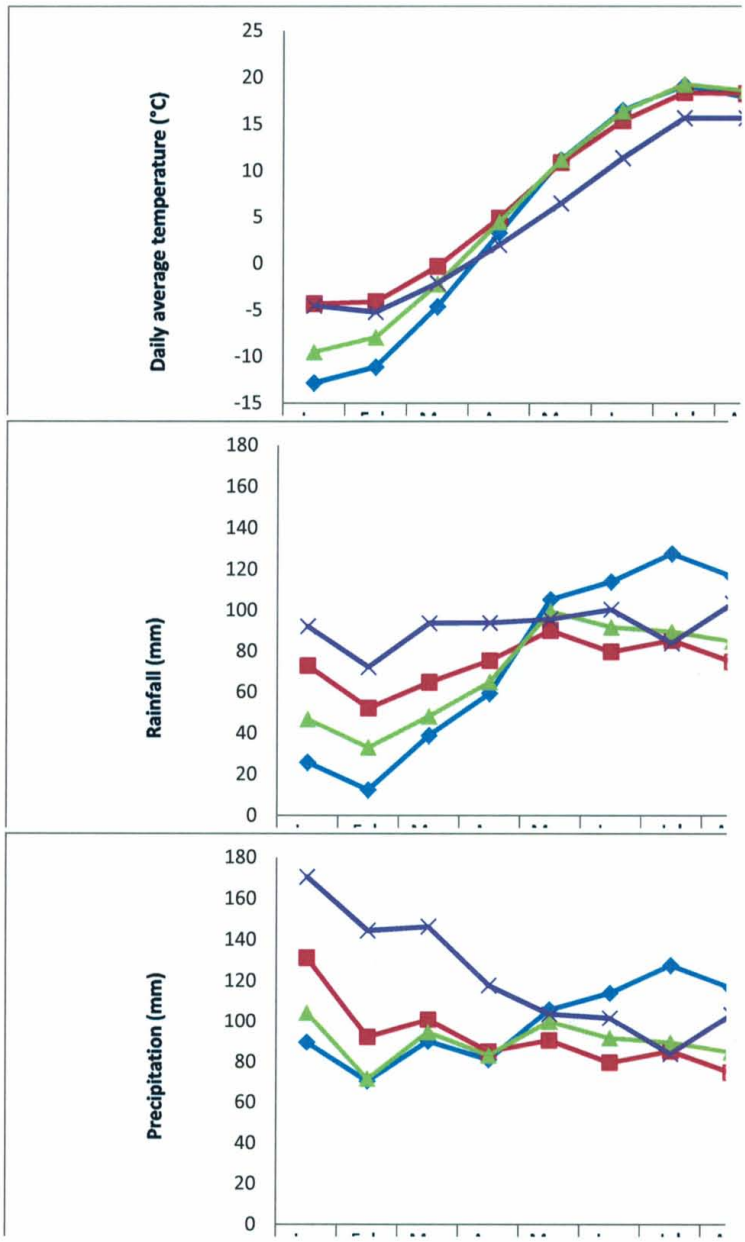

FIGURE 1.5 Summary of average Climate Normals 1971-2000 for four weather stations located in Quebec and Atlantic Canada. Data from National Climate Data and Information Archive (Retrieved June 9, 2010). http://www.climate.weatheroffice.gc.ca/climate_normals/index_e.html Date Modified: 2010-03-18.

1.6.2 Comparison among Populations

The fitness of individual populations is assumed to be under selection pressure created by factors that vary among residential lawns. These factors include vegetation, competition, predators and parasitoids, soil conditions, shading and physical exposure to weather. These factors are not specifically evaluated in this study, as this research focuses primarily on identifying differences in life strategies among local populations. Although each of the sampling sites in this study are characterized by turf hosting populations of *B. leucopterus hirtus*, each site is assumed to be unique, as each is influenced by a different set of environmental pressures because of geographic location, soil type, exposure to sun and rain, and differing patterns in use. It was the goal of this thesis to determine whether those environmental influences have been sufficient to promote measurable differences in key life history traits in *B. leucopterus hirtus* and to offer some preliminary suggestions for explaining the differences observed.

Although 'regional population groups' are often difficult to define (Srivastava 1999), and often arbitrarily assigned (Caley and Schluter 1997), they are generally assumed to be influenced through variation in climate and other large scale influences in space and time (Hillebrand and Blanckner 2002). In designing this research project, it was assumed that climate at the local scale, within a few kilometer radius, would be less variable among sites than at a regional scale, defined in this study as sites separated by 100 km or more. Four *regional populations* were selected: 1. St. John's, Newfoundland and Labrador, 2. The Annapolis Valley, Nova Scotia, 3. Fredericton and Saint John, New Brunswick, and 4. Quebec City, Quebec. (Appendix A). For convenience thereafter in

this thesis, the four regions were referred to by province within which each of these study locations occur. This is not meant to imply that environmental conditions conform to provincial boundaries, but rather, this is a simple convention to identify the clusters of samples taken which happen to fall within different provinces.

1.7 Thesis Outline

This thesis explored variation in three measures of life history (phenology, morphometrics and host choice) to compare variation in life history strategy of *B. leucopterus hirtus* populations at a local and regional level in Quebec and Atlantic Canada, with emphasis on populations in St. John's, Newfoundland and Labrador. The purpose of this thesis is to gain an understanding of the geographic variation in *B. leucopterus hirtus* biology for the purpose of adding needed geographic and/or population specificity to ecologically based management approaches of this pest. By using Southwood's habitat templet model for understanding environmentally induced change, the author hopes to place findings in a sound theoretical framework. This study should further determine at what scale the impact of environmental variation is manifested through changes in life history, morphology and host choice. As with all pest species, truly effective management is accomplished through knowledge of the pest's capacity to adapt to new environments and expand into novel habitats, and adapt strategies for thriving under potential climate change. This study aims at elucidating multiple indicators of variation among populations at two scales and, in the case of host choice, at multiple points in the insect's phenology.

1.7.1 Phenology

Many environmental pressures influence populations through natural selection and phenotypic plasticity (Gotthard and Nylin 1995, Nylin and Gotthard 1998, West-Eberhard 2003, Nylin et al. 2004, Whitman and Ananthakrishnan 2009). Environmental influences

on population dynamics are numerous but often linked, resulting in complex responses involving a series of fitness tradeoffs. In insects, these complex responses include changes in voltinism, growth rate, dispersal, diapause and many other measures of life strategy (Danks 1994, 2006, 2007, Tauber et al. 1986) that lead to adaptations and unique life histories across geographic and climatically diverse ranges.

Changes in the timing of key life events for the purpose of synchronizing with host plant phenology, photoperiod and optimal temperatures, are commonly observed in many insects, particularly in seasonal environments (Danks 2006, Danks 2007). Phenological variation related to environmental conditions has been observed in a large number of insect Orders, including: Lepidoptera (Leimar 1996, Gotthard 1998, Friberg and Wiklund 2010), Coleoptera (Ishihara 1998, Sota 1994), Orthoptera (López et al. 2007), Trichoptera (Shama and Robinson 2009), Diptera (Demont and Blanckenhorn 2008, Ragland and Kingsolver 2008), Odonata (de Block and Stoks 2004) Hymenoptera (Traoré et al. 2006) and Hemiptera (Leslie 1990, Baldwin and Dingle 1986). In an example with a closely related species, the large milkweed bug, *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae), samples were collected from six locations ranging from tropical islands to mid latitude forests. Among the six locations, the insects were found to vary among populations in age at first reproduction, clutch size and rate of egg production (Leslie 1990). This Hemipteran research has clearly shown the capacity of insects to adapt their life history under a given environmental regime in order to optimize fitness, and suggests that variation in the phenology of *B. leucopterus hirtus* under a variety of stressors is also possible.

To quantify local variation in phenology of *B. leucopterus hirtus*, life history data were collected from St. John's, NL over two years. Variation among populations in St. John's was evaluated using phenological comparison among ten local sites, measurements of egg development threshold and rate of development. This data was then compared with previously published phenological records from populations in different locations within the United States and Canada. The results of this work are presented in *Chapter 2: Local and Regional Level Variation in Phenology of Blissus leucopterus hirtus Montandon (Hemiptera: Blissidae)* and evaluated with regard to the need for regional and/or population specific management strategies.

1.7.2 Morphology

1.7.2.1 Size

Morphometrics was one of the first methods used to detect diversity among populations within species (Daly 1985). The morphometric characteristics of an individual, population or species can be interpreted within a biological, ecological and evolutionary context to better understand the life strategy and adaptive potential of the organism. Morphometrics have been particularly well researched in insects, which are easily collected and measured due to their rigid exoskeletons (Daly 1985, Statzner et al. 2001). Since Johan Christian Fabricius first related structures of insects to the insect's function in the environment (referenced in Tuxen 1967, Statzner et al. 2001), morphometrics has proven to be a powerful tool in detecting variation among individuals and populations. This subsequently provides a better understanding of the life strategy

and adaptive potential of the organism in the field of ecological and evolutionary entomology (Daly 1985, Statzner et al. 2001). As an example, size is frequently linked to growth rate (Nylin and Gotthard 1998) and female fecundity (Honěk 1993). There is considerable potential for comparative morphometric variation to provide insight into environmental influences and subsequent adaptation by insects.

As alluded to above, the relationship between size, growth rate and environmental conditions is complex and generally involves a series of trade-offs (Danks 2006, Danks 2007). Small size is typically considered a tradeoff for shorter development time and/or faster growth rate (Nylin and Gotthard 1998) and may be further influenced by factors including temperature (Sibley and Atkinson 1994) and food supply (Colbo and Porter 1979) and their interactive effects (Colbo and Porter 1981). Therefore, differences in mean size of insects among populations likely indicate differences in the environmental influences to which each population has responded, and should lead to insight on the relative fitness of individuals within these populations under a given habitat. Detection of morphometric variation among populations of *B. leucopterus hirtus* would confirm variation in selective forces on individual groups, and provide a foundation for elucidating specific environmental influences that impact individual size and fitness.

1.7.2.2 Wing Form

The dimorphic wing form found in many insect species is easily distinguishable and can offer additional insight into adaptive strategies in novel environments (Roff 1986). The most commonly accepted 'rule of thumb' for wing form is that short wing

individuals are selected when conditions are optimal in the present location, while long wing individuals are generally produced under conditions where it is in the best interest of the insect to move elsewhere for either resources or mating opportunities (Harrison 1980). Previous papers on *B. leucopterus hirtus* (Mailloux and Streu 1981) speculated that a high incidence of macropterous wing form reflected environmental pressures promoting dispersal, but a comprehensive comparison of wing forms among populations of *B. leucopterus hirtus* is lacking. Detection of variation in wing morphism ratios among populations of this insect would indicate a high likelihood that populations are experiencing different pressures to establish or disperse. Because this thesis examined populations from a number of local and regionally separated areas, it was expected that differences in establishment would be reflected through wing form ratios. Being able to relate wing ratio to density, age of population, host plant quality and site-specific biotic and abiotic features is the ultimate goal and would further the understanding of this pest's ecology.

Using morphometrics as a tool to compare variation among local and regional populations, *Chapter 3: Local and Regional Level Variation in Sex Ratio, Wing Morph and Mensural Characters in Blissus leucopterus hirtus Montandon (Hemiptera: Blissidae)* summarizes results comparing sex and wing ratios among local and regional populations. Additional measurements were collected to compare morphometric characters and to determine the level of intra and inter-population differences to identify the scale at which variation occurs.

1.7.3 Host Choice Behaviour

The spatial patterns that govern herbivore-plant host relationships are of great interest to ecologists and pest managers because of the potential for specialization and eventual speciation by insects (Gotthard et al. 2004, Nosil et al. 2005). Variation in host choice as a result of plasticity may eventually lead to the formation of new colonies (Futuyma and Peterson 1985) and colonization of new hosts may directly influence the phenology and morphology of herbivore populations as the insect's life cycle becomes increasingly synchronized with that of the plant (Vanbergen et al. 2003). Because host choice (preference in feeding, oviposition sites or basking sites) can influence size, developmental time and growth rate (Grossmueller and Lederhouse 1985, Nylin and Gotthard 1998), it is an important indicator in comparison of life strategies among insect populations. Difference in behaviour among insect populations is a third marker of variation, along with phenology and morphometrics that may be influences as a result of specific environmental pressures (Scriber 1994).

Because insects are poikilothermic, their degree of activity is especially dependent on the external environment. Weather and climate therefore have important implications for insect behaviour and can also affect the feeding rates and food choice of insects (Schoonhoven et al. 2005). Host plant selection can also be affected by the insect's tendency to optimize body temperature by changing diurnal conditions throughout the day. For example, the black desert grasshopper, *Taeniopoda eques* Burmeister (Orthoptera: Acrididae), roosts on elevated plant parts on sunny days, causing the grasshopper to consume certain parts of the plant it would not normally encounter on

cloudy days (Whitman 1987). Another example is the tiger swallowtail butterfly, *Papilio glaucus* L. (Lepidoptera: Papilionidae), that oviposits preferentially on the tips of sun-exposed branches to optimize daily temperature potential (Grossmueller and Lederhouse 1985). These examples demonstrate that the prevailing weather patterns in a specific area may have an important influence on the behaviour of insects with respect to host selection. Geographical patterns in climate and vegetation may result in variable host choice at a regional scale as a result of available plant hosts, plant structure and weather and therefore play an important role in host selection and utilization.

At a smaller scale, previous work on *B. leucopterus hirtus* shows that this insect is capable of discriminating among hosts, including cultivars of the same species, even at a micro-geographic scale (i.e. among tillers (stems of grass)) (Mathias et al. 1990, Carrière et al. 1998). The ability of these insects to discriminate among infected and non-infected grasses suggests that variation in host selection can occur not only in a regional context, but also on a localized scale as a result of intra-lawn and inter-plant variation. Awareness of the potential for variation at this scale is essential to the design, interpretation and application of future host choice studies because of the need to incorporate the potential for variation among lawns, sports fields and golf courses, where, depending on the care and maintenance of the turf, the landscape may be quite varied.

While variability in the selection of host as a result of extrinsic determinants (host availability, quality and weather) is an main component of this research, it is also important to explore the compounding influence of phenology and group dynamics (density) on host choice. Life history stage of an insect can affect host choice because of

changing dietary needs throughout ontogeny. For example, as caterpillars of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: *Lymantriidae*) developed from 3rd to 6th instar, they exhibited increased preference for diet cubes with high lipid concentration in conjunction with decreased preference for cubes with high protein concentration (Stockhoff 1993). Morphological characteristics related to phenology may also influence host choice with age. The 1st, 2nd and 3rd instar nymphs of *Oncopeltus fasciatus* are unable to access the seeds of common milkweed, *Asclepias syriaca* L. (Apocynaceae: Asclepiadoideae), because their short proboscis is incapable of penetrating the surrounding pod (Ralph 1976). However, in the same experiment it was found that feeding in larger groups produced lower mortality on feeding locations other than on seeds. A potential explanation is increased ingestion efficiency through the mixing of saliva, making group size an important host choice factor.

Group size is a relevant factor in the host choice of *B. leucopterus hirtus* because of variation in population size that may occur as a result of environmental pressures on the population. For example, under conditions of population stress or recolonization as a result of varying habitat conditions, there may be fewer insects participating in group feeding behaviour. Previous studies in Hemiptera have shown that group size may influence feeding efficacy and individual mortality (Bongers and Eggermann 1971, Ralph 1976). It is important to identify any variation in host choice that may occur as a result of lower density under these conditions, so that potential hosts may be identified under a variety of conditions.

Chapter 4: Host Choice Behaviour of Blissus leucopterus hirtus Montandon (Hemiptera: Blissidae) in Relation to Geographic Distribution, Insect Age and Insect Group Size reports on the level of variation in host choice behaviour among locally and regionally separated populations of *B. leucopterus hirtus.* Studies were conducted using a series of multi-choice experiments conducted in small arenas. Using similar techniques, additional experiments explored whether host choice changed with insect age or group size. The results of this chapter suggested that multiple factors were responsible for host choice in *B. leucopterus hirtus*, including phenology, confirming the need for additional context in host choice studies with this insect.

1.8 Summary

Blissus leucopterus hirtus is a well-known and economically important pest species. However, there is little information on its ecology, particularly as it relates to differences among populations. This research aims to identify potential variation in the phenology, morphology and host preference of *B. leucopterus hirtus*, at both the local and regional scale. In the case of host choice, consideration was also be given to life stage and group size. The results of this research should provide improved information for ecologically based control strategies that rely on phenology, morphology and host choice to manage this pest. If this thesis is successful in identifying variation among these key indicators of life strategy, turf managers should be better able to 1. conduct appropriately timed pesticide application based on more accurate predictions of 2nd instar appearance, particularly in St. John's NL, where a two year study should elucidate locally specific phenological patterns 2. use indicators such as sex ratio, wing morph and size to estimate

the relative level of establishment, potential for dispersal, and level of fecundity among populations, and 3. plant suitable host vegetation to mitigate pest damage, as well use knowledge of plant preferences and aggregation to locate and target *B. leucopterus hirtus* as part of an integrated management strategy that may use alternate method of control, including vacuuming. With so little know about the basic biology and ecology of this serious turfgrass pest, this thesis takes the necessary steps to establish a framework for environmental influence on this species within the context of Southwood's habitat templet model.

Literature Cited

Agriculture and Agri-Food Canada (AAFC). 2005. News Release: Keeping the greens green. 18 October 2005. Beloeil, Quebec, Canada.

Ahmad, T. R., K. P. Pruess, and S. D. Kindler. 1984. Non-crop grasses as hosts for the chinch bug, *Blissus leucopterus leucopterus* (Say) (Hemiptera: Lygaeidae). Journal of the Kansas Entomological Society 57:17-20.

Anderson, W. G., T. M. Heng-Moss, F. P. Baxendale, L. M. Baird, G. Sarath, and L. Higley. 2006. Chinch bug (Hemiptera: Blissidae) mouthpart morphology, probing frequencies, and locations on resistant and susceptible germplasm. Journal of Economic Entomology 99:212-221.

Baker, P. B., R. H. Ratcliffe, and A. L. Steinhauer. 1981. Tolerance to hairy chinch bug feeding in Kentucky bluegrass. Environmental Entomology 10:153-157.

Baldwin, J. D., and H. Dingle. 1986. Geographic variation in the effects of temperature on life-history traits in the large milkweed bug *Oncopeltus fasciatus*. Oecologia 69:64-71.

Banfield, C.E. 1983. Climate. Pages 37-106 *in* G.R. South, editor. Biogeography and Ecology of the Island of Newfoundland. Kluwer Boston, Inc., Hingham, Massachusetts, USA.

Barber, H. G. 1918. A new species of *Leptoglossus*: a new *Blissus* and varieties. Bulletin of the Brooklyn Entomological Society 13:36.

Bongers, J., and W. Eggermann. 1971. Der Einfluß des Subsozialverhaltens der spezialisierten Samensauger *Oncopeltus fasciatus* Dall. und *Dysdercus fasciatus* Sign, auf ihre Ernährung. Oecologia (Berl.) 6:293-302.

Bourassa, S., J. Brodeur, and Y. Carrière. 2007. Endophyte-grass complexes and the relationship between feeding preference and performance in a grass herbivore. Entomologia Experimentalis et Applicata 124:221-228.

Caley, J. M., and D. Schluter. 1997. The relationship between local and regional diversity. Ecology 78:70-80.

Carrière, Y., A. Bouchard, S. Bourassa, and J. Brodeur. 1998. Effect of endophyte incidence in perennial ryegrass on distribution, host choice, and performance of the hairy chinch bug (Hemiptera: Lygaeidae). Journal of Economic Entomology 91:324-328.

Colbo, M. H., and G. N. Porter. 1979. Effects of the food supply on the life history of Simuliidae (Diptera). Canadian Journal of Zoology 57:301-306.

Colbo, M. H., and G. N. Porter. 1981. The interaction of rearing temperature and food supply on the life history of two species of Simuliidae (Diptera). Canadian Journal of Zoology 59:158-163

Crnokrak, P., and D. A. Roff. 2002. Trade-offs to flight capacity in *Gryllus firmus*: the influences of whole-organism respiration rate on fitness. Journal of Evolutionary Biology 15:388-398.

Daly, H. V. 1985. Insect morphometrics. Annual Review of Entomology 30:415-438.

Danks, H. V. 1994. Diversity and integration of life-cycle controls in insects. Pages 5-40 *in* H. Danks, editor. Life cycle polymorphism: theory, evolution and ecological consequences for seasonality and diapause control. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Danks, H. V. 2006. Key themes in the study of seasonal adaptations in insects II. Life-cycle patterns. Applied Entomology and Zoology 41:1-13.

Danks, H. V. 2007. The elements of seasonal adaptations in insects. Canadian Entomologist 139:1-44.

de Block, M., and R. Stoks. 2004. Life history variation in relation to time constraints in a damselfly. Oecologica 140:68-75.

Demont, M., and W. U. Blanckenhorn. 2008. Genetic differentiation in diapause response along a latitudinal cline in European yellow dung fly populations. Ecological Entomology 33:197-2001.

Denno, R. F., K. L. Olmstead, and E. S. McCloud. 1989. Reproductive cost of flight capacity: a comparison of life history traits in wing dimorphic planthoppers. Ecological Entomology 14:31-44.

Diamond, S. E., and J. G. Kingsolver. 2010. Fitness consequences of host plant choice: a field experiment. Oikos 119:542-550.

Eickhoff, T. E., F. P. Baxendale, and T. M. Heng-Moss. 2006. Host preference of the chinch bug, *Blissus occiduus*. Journal of Insect Science 6:1-6.

Eickhoff, T. E., F. P. Baxendale, T. M. Heng-Moss, and E. E. Blankenship. 2004. Turfgrass, crop, and weed hosts of *Blissus occiduus* (Hemiptera: Lygaeidae). Journal of Economic Entomology 97:63-73.

Emmons, R. D. 2000. Turfgrass Science and Management, 3rd ed. Delmar Publishing Inc., Albany, New York, USA.

Fitch, A. 1856. Chinch Bug. Transactions of the New York State Agricultural Society. 15:509-526.

Friberg, M., and C. Wiklund. 2010. Host-plant-induced larval decision-making in a habitat/host-plant generalist butterfly. Ecology 91:15-21.

Fujisaki, K. 1993. Genetic correlation of wing polymorphism between females and males in the oriental chinch bug, *Cavelerius saccharivorus* Okajima (Heteroptera: Lygaeidae). Researches on Population Ecology 35:317-334.

Futuyma, D. J., and S. C. Peterson. 1985. Genetic variation in the use of resources by insects. Annual Review of Entomology 30:217-238.

Gotthard, K. 1998. Life history plasticity in the satyrine butterfly *Lasiommata petropolitana*: Investigating an adaptive reaction norm. Journal of Evolutionary Biology 11:21-39.

Gotthard, K., N. Margraf, and M. Rehier. 2004. Geographic variation in oviposition choice of a leaf beetle: the relationship between host plant ranking, specificity, and motivation. Entomologia Experimentalis et Applicata 110:217-224.

Gotthard, K., and S. Nylin. 1995. Adaptive plasticity and plasticity as an adaption: a selective review of plasticity in animal morphology and life history. Oikos 74:3-17.

Grossmueller, D. W., and R. C. Lederhouse. 1985. Oviposition site selection: an aid to rapid growth and development in the tiger swallowtail butterfly, *Papilo glaucus*. Oecologia 66:68-73.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annual Review of Entomology 11:95-118.

Headlee, T. J., and J. Walker. 1913. The chinch bug (*Blissus leucopterus* Say). Kansas State Agricultural College Agriculture Experiment Station Bulletin 91:287-353.

Henry, T. J. 1997. Phylogenetic analysis of family groups within the infraorder Pentatomomorpha (Hemiptera: Heteroptera), with emphasis on the Lygaeoidea. Annals of the Entomological Society of America 90:275-301.

Henry, T. J., and R. C. Froeschner. 1988. Catalog of the Heteroptera, or True Bugs, of Canada and the Continental United States. E. J. Brill, New York, New York, USA.

Hillebrand, H., and T. Blanckner. 2002. Regional and local impact on species diversity – from pattern to processes. Oecologia 132:479-491.

Honěk, A. 1993. Intra-specific variation in body size and fecundity in insects: a general relationship. Oikos 66:483-492.

Ishihara, M. 1998. Geographical variation in insect developmental period: Effect of host plant phenology on the life cycle of the bruchid seed feeder *Kytorhinus sharpianus*. Entomologia Experimentalis et Applicata 87:311-319.

Johnson, J. P. 1941. Control of the hairy chinch bug. Bulletin - Connecticut Agricultural Experiment Station 445:372-373.

Lamp, W. O., and T. O. Holtzer. 1980. Distribution of overwintering chinch bugs *Blissus leucopterus leucopterus* (Hemiptera: Lygaeidae). Journal of the Kansas Entomological Society 53:320-324.

Langellotto, G. A., and R. F. Denno. 2001. Benefits of dispersal in patchy environments: mate location by males of a wing-dimorphic insect. Ecology 82:1870-1878.

Langellotto, G. A., R. F. Denno, and J. R. Ott. 2000. A trade-off between flight capability and reproduction in males of a wing-dimorphic insect. Ecology 81:865-875.

Leimar, O. 1996. Life history plasticity: Influence of photoperiod on growth and development in the common blue butterfly. Oikos 76:228-234.

Leonard, D. E. 1966. Biosystematics of the *leucopterus* complex of the genus *Blissus* (Hemiptera: Lygaeidae). Bulletin - Connecticut Agricultural Experiment Station 677:1-47.

Leonard, D. E. 1968. A revision of the genus *Blissus* (Heteroptera: Lygaeidae) in Eastern North America. Annals of the Entomological Society of America 61:239-250.

Leslie, J. F. 1990. Geographical and genetic structure of life history variation in milkweed bugs (Hemiptera: Lygaeidae: *Oncopeltus*). Evolution 44:295-304.

Lintner, J. A. 1883. The chinch-bug in New York. Science 2:540.

Liu, H. J., and F. L. McEwen. 1979. The use of temperature accumulations and sequential sampling in predicting damaging populations of *Blissus leucopterus hirtus.* Environmental Entomology 8:512-515.

López, H., M. Nogales, and E. Morales. 2007. Habitat use and phenology of the large insular endemic grasshopper *Acrostira euphorbiae* (Orthoptera: Pamphagidae). Bulletin of Entomological Research 97:117-127.

Luginbill, P. 1922. Bionomics of the chinch bug. USDA Bureau Entomology Bulletin 1016:1-14.

Mailloux, G., and H. T. Streu. 1981. Population biology of the hairy chinch bug (*B. leucopterus hirtus,* Montandon: Hemiptera: Lygaeidae). Annals of the Entomological Society of Quebec 26:51-90.

Mailloux, G., and H. T. Streu. 1982. Spatial pattern of hairy chinch bug (*B. leucopterus hirtus* Montandon: Hemiptera: Lygaeidae) populations in turfgrass. Annals of the Entomological Society of Quebec 27:111-131.

Mathias, J. K., R. H. Ratcliffe, and J. L. Hellman. 1990. Association of an endophytic fungus in perennial ryegrass and resistance to the hairy chinch bug (Hemiptera: Lygaeidae). Journal of Economic Entomology 83:1640-1646.

Maxwell, K. E., and G. F. MacLeod. 1936. Experimental studies of the hairy chinch bug. Journal of Economic Entomology 29:339-343.

Meehan, M., and G. Wilde. 1989. Screening for sorghum line and hybrid resistance to chinch bug (Hemiptera: Lygaeidae) in the greenhouse and growth chamber. Journal of Economic Entomology 82:616-620.

Mize, T. W., and G. Wilde. 1986. New grain sorghum sources of antibiosis to the chinch bug (Heteroptera: Lygaeidae). Journal of Economic Entomology 79:176-180.

Mize, T. W., G. Wilde and M. T. Smith. 1980. Chemical control of the chinch bug and greenbug on seedling sorghum with seed, soil and foliar treatment. Journal of the Kansas Entomological Society 67:116-125.

Montandon, A. L. 1893. Lygaeidae exotiques. Notes et description d'especes nouvelles. Annals de la Societé Entomologique de Belge 37:399.

Negrón, J. F., and T. J. Riley. 1985. Effect of chinch bug (Heteroptera: Lygaeidae) feeding in seedling field corn. Journal of Economic Entomology 78:1370-1372.

Negrón, J. F., and T. J. Riley. 1990. Long-term effects of chinch bug (Hemiptera: Heteroptera: Lygaeidae) feeding on corn. Journal of Economic Entomology 83:618-620.

Norris, R. F., E. P. Caswell-Chen, and M. Kogan. 2002. Chapter 4: Ecosystems and pest organisms. Pages 66-89 *in* Concepts in integrated pest management. Prentice Hall, Upper Saddle River, N. J., USA.

Nosil, P., C. P. Sandoval, and B. J. Crespi. 2005. The evolution of host preference in allopatric vs. parapatric populations of *Timema cristinae* walking sticks. Journal of Evolutionary Biology 19:929-942.

Nylin, S., and K. Gotthard. 1998. Plasticity in life history traits. Annual Review of Entomology 43:63-83.

Nylin, S., K. Gotthard, and G. H. Nygren. 2004. Seasonal plasticity, host plants, and the origin of butterfly biodiversity. Pages 11-137 *in* Fellowes, M. D. E, G. J. Holloway, and J. Rolff, editors. Insect evolutionary ecology. CABI Publishing, Oxon, UK.

Packard, C. M. 1937. How to fight the chinch bug. Farmer's Bulletin: U.S. Department of Agriculture 1780:1-21.

Polivka, J. B. 1963. Control of hairy chinch bug, *Blissus leucopterus hirtus*, Mont., in Ohio. Ohio Agricultural Experiment Station Research Circular 122.

Potter, D. A. 1998. Destructive turfgrass insects: biology, diagnosis and control. Ann Arbor Press, Chelsea, Michigan, USA.

Pöyry, J., R. Leinonen, G. Soderman, M. Nieminen, R. Heikkinen, and T. Carter. 2011. Climate-induced increase of moth multivoltinism in boreal regions. Global Ecology and Biogeography 20:289-298.

Ragland, G. J., and J. G. Kingsolver. 2008. Evolution of thermotolerance in seasonal environments: the effects of annual temperature variation and life history timing in *Wyeomyia smithii*. Evolution 62:1345-1357.

Ralph, C. P. 1976. Natural Food Requirements of the Large Milkweed Bug, *Oncopeltus fasciatus* (Hemiptera: Lygaeidae), and Their Relation to Gregariousness and Host Plant Morphology Oecologia 26:157-175.

Richmond, D. S., and Shetlar, D. J. 2000. Hairy chinch bug (Hemiptera: Lygaeidae) damage, population density, and movement in relation to the incidence of perennial ryegrass infected by *Neotyphodium* endophytes. Journal of Economic Entomology 93:1167-1172.

Roff, D. A. 1986. The evolution of wing dimorphism in insects. Evolution 40:1009-1020.

Roff, D. A. 1994. Habitat persistence and the evolution of wing dimorphism in insects. The American Naturalist 144:772-798.

Schoonhoven, L., van Loon, J. J. A., and M. Dicke. 2005. Insect-Plant Biology. Oxford University Press, Oxford.

Scriber, J. M. 1994. A new 'cold pocket' hypothesis to explain local host preference shifts in *Papilio Canadensis*. Entomologia Experimentalis et Applicata 80:315-319.

Sears, M. K., G. McEwen, G. Ritcey, and R. R. McGraw. 1980. Evaluation of insecticides for the control of hairy chinch bugs (Hemiptera: Lygaeidae) in Ontario lawns. Proceedings of the Entomological Society of Ontario 111:13-20.

Shafiei, M., Moczek, A. P. and H.F.Hijhout. 2001. Food availability controls the onset of metamorphosis in the dung beetle Onthophagus Taurus (Coleoptera: Scarabaeidae). Physiological Entomology 26:173-181.

Shama, L. N. S., and C. T. Robinson. 2009. Microgeographic life history variation in the alpine caddisfly: plasticity in response to seasonal time constraints. Freshwater Biology 54:150-164.

Sibley, R. M., and D. Atkinson. 1994. How rearing temperature affects optimal adult size in ectotherms. Functional Ecology 8:*486-493.*

Slater, J. A. 1976. Monocots and chinch bugs: a study of host plant relationships in the lygaeid subfamily Blissinae (Hemiptera: Lygaeidae). Biotropica 8:143-165.

Solbreck, C. 1978. Migration, diapause, and direct development as alternative life strategies in a seed bug, *Neacoryphus bicrucis*. Pages 195-217 *in* H. Dingle editor. Evolution of insect migration and diapause. Springer-Verlag, New York, New York, USA.

Sota, T. 1994. Variation of carabid life cycles along climatic gradients: an adaptive perspective for life-history evolution under adverse conditions. Pages 99-112 *in* H. V. Danks, editor. Insect life-cycle polymorphisms. Kluwer Academic, Dordrecht, Netherlands.

Southwood, T. R. E. 1962. Migration of terrestrial arthropods in relation to habitat. Biological Reviews 37:171-214.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology 46:336-365.

Srivastava, D. S. 1999. Using local–regional richness plots to test for species saturation: pitfalls and potentials. Journal of Animal Ecology 68:1-16.

Statzner, B., A. G. Hildrew, and V. H. Resh. 2001. Species traits and environmental constraints: entomological research and the history of ecological theory. Annual Review of Entomology 46:291-316.

Stockhoff, B. A. 1993. Ontogenetic change in the dietary selection for protein and lipid by gypsy month larvae. Journal of Insect Physiology 39:677-686

Stuart, J., G. Wilde, and J. H. Hatchett. 1985. Chinch-bug (Heteroptera: Lygaeidae) reproduction, development, and feeding preferences on various wheat cultivars and genetic sources. Economic Entomology 79:664-667.

Tashiro, H. 1987. Turfgrass insects of the United States and Canada. Cornell University Press, New York, New York, USA.

Tauber, M. J., C. A. Tauber, and S. Masaki, 1986. Seasonal Adaptations of Insects. Oxford University Press, Oxford, UK.

Traoré, L., J-G Pilon, F. Fournier, and G. Boivin. 2006. Adaptation of the development process of *Anaphes victus* (Hymenoptera: Mymaridae) to local climatic conditions across North America. Annals of the Entomological Society of America 99:1121-1126.

Tuxen, S. L. 1967. The Entomologist, J. C. Fabricius. Annual Review of Entomology 12:1-15.

van Duzee, E. P. 1886. Occurrence of the chinch bug (*Blissus leucopterus*) at Buffalo, N.Y.. Canadian Entomologist 18:209-210.

Vanbergen, A. J., B. Raymond, I. S. K. Pearce, A. D. Watt, R. S. Halls, and S. E. Hartley. 2003. Host shifting by *Operophtera brumata* into novel environments leads to population differentiation in life history traits. Ecological Entomology 28:604-612.

Vittum, P., M. Villani, and H. Tashiro, 1999. Turfgrass insects of the United States and Canada, 2nd ed. Cornell University Press, New York, USA.

Wellwood, A., G. Nickerson, and J. Wetmore, 2002. Hairy chinch bug survey, demonstration and monitoring in New Brunswick, 2002. New Brunswick Department of Agriculture, Fisheries and Aquaculture and New Brunswick Horticultural Trades Association, Fredericton, New Brunswick, Canada.

Wennström, A. L. N. Hjulström, J. Hjältén, and R. Julkunen-Tiitto. 2010. Mother really knows best: host choice of adult phytophagous insect females reflects a within-host variation in suitability as larval food. Chemoecology 20:35-42.

West-Eberhard, M. J. 2003. Developmental Plasticity and Evolution. Oxford University Press Inc. New York, New York, USA.

Whitman, D. W. 1987. Thermoregulation and daily activity patterns in a black desert grasshopper, *Taeniopoda eques. Animal Behaviour* 35:1814-1826.

Whitman, D. W., and T. N. Ananthakrisnan. 2009. Phenotypic plasticity of insects: mechanisms and consequences. Science Publishers, Enfield, New Hampshire, USA.

Wilde, G. 1997. Effect of imidicloprid seed treatment and planting time applications of insecticides on chinch bug (Hemiptera: Lygaeidae) and resulting yields of sorghum. Journal of Agricultural Entomology 14:385-391.

Yue, Q., J. Johnson-Cicalese, T. J. Gianfagna, and W. A. Meyer. 2000. Alkaloid production and chinch bug resistance in endophyte-inoculated chewings and strong creeping red fescues. Journal of Chemical Ecology 279-292.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42:207-30.

Zera, A. J., and S. Mole. 1994. The physiological costs of flight capacity in wing-dimorphic crickets. Researches on Population Ecology 36:151-156.

Zera, A. J., and K. C. Tiebel. 1988. Brachypterizing effect of group rearing, juvenile hormone III and methoprene in the wing-dimorphic cricket, *Gryllus rubens*. Journal of insect Physiology 34:489-498.

Chapter 2: Local and Regional Level Variation in Phenology of *Blissus leucopterus hirtus* Montandon (Hemiptera: Blissidae)

2.1 Introduction

2.1.1 Phenology and the Environment

Phenology is a life history trait defined as the timing of naturally occurring events in an organism's life cycle. Intricate knowledge of insect pest life cycles assists pest managers in monitoring and predicting key life stages for more precise timing of control measures. The practice of incorporating this knowledge into environmentally responsible control measures is called Integrated Pest Management (IPM). It is a multi-dimensional based approach requiring a thorough understanding of insect population strategy and life history (Norris et al. 2002). Phenology of insects is largely influenced by the natural environment (Denno and Dingle 1981, Masaki and Wipking 1994, Danks 1994, Nylin and Gotthard 1998), which exerts selective pressure on insect populations, causing an adaptive response in which the life cycle is optimized for the prevailing conditions. The habitat templet model (Southwood 1977) predicts that insects adapt a life history strategy that accounts for the current and future potential conditions at both the current location and elsewhere. This may lead to a spectrum of phenological expressions within a single species.

The association between phenological variation and environmental conditions has been recognized within a range of Orders including: Lepidoptera (Leimar 1996, Gotthard 1998, Friberg and Wiklund 2010), Coleoptera (Ishihara 1998, Sota 1994), Orthoptera (López et al. 2007), Trichoptera (Shama and Robinson 2009), Diptera (Demont and Blanckenhorn 2008, Ragland and Kingsolver 2008), Odonata (de Block and Stoks 2004), Hymenoptera (Traoré et al. 2006) and Hemiptera (Leslie 1990). Dominant habitat characteristics including seasonality, temperature, precipitation and photoperiod are often linked, resulting in a complex response by an insect that involves a series of tradeoffs in voltinism, growth rate, dispersal and many other measures of life strategy (Danks 1994, 2006, 2007, Tauber et al. 1986). Because these environmental factors can vary across geographic ranges, knowledge and incorporation of these differences is an important component of developing a regionally based integrated pest management approach.

2.1.2 Biology and Ecology of *Blissus leucopterus hirtus*

In part because pest insect species tend to have broad habitat and geographic distributions (Showers 1981), phenological variation across habitats and ranges is common. *Blissus leucopterus hirtus* Montandon (Hemiptera: Blissidae) (hairy chinch bug), is a serious turfgrass and crop pest in Quebec and Atlantic Canada, and the northeastern United States (Leonard 1966, Vittum et al. 1999, Potter 1998). It is widely distributed in Ontario, Quebec, New Brunswick, Nova Scotia, Prince Edward Island and also on the island of Newfoundland where, in the past decade, it has become a serious turfgrass pest. The species was first recorded in Newfoundland by Brown (1949). Whether its presence on the island is the result of an introduction from the mainland, or natural dispersal by wind and only recorded due to better faunal surveys conducted by the

Government of Canada is unknown. However a recent survey, conducted as part of this study, has shown *B. leucopterus hirtus* is widely distributed across Newfoundland (Appendix B).

Despite the economic interest in this insect as a pest of turfgrass, there have been only a few locally based studies that have documented the complete life history of *B. leucopterus hirtus* (Mailloux and Streu 1981, Wellwood et al. 2002). Supplemental information comes from partial studies of this insect's life cycle (Liu and McEwen 1979, Sears et al. 1980). Based on these individual reports, there is strong evidence that phenological variation exists among populations from various parts of the insect's range. Variability in the timing of appearance of instars has been recorded at the regional (provincial/state) level (Potter 1998) and between communities fewer than 100 km apart (Wellwood et al. 2002). Bivoltine populations of *B. leucopterus hirtus* have been observed in the American states of New Jersey, (Mailloux and Streu 1981), New York (Maxwell and MacLeod 1936), Connecticut (Johnson 1941) and Ohio (Polivka 1963). Further north in Ontario, Canada, a univoltine population has also been recorded (Liu and McEwen 1979). Researchers in New Brunswick reported one complete cohort per year with a second cohort initiating, but not completing development (Wellwood et al. 2002).

Because of the local nature of each of these studies, there was no attempt to evaluate the relationship between phenology and geographic or climate patterns. From these studies it is not clear what selection pressures have resulted in reported differences in life cycle, although the pattern of decreasing voltinism at northern latitudes suggests temperature and/or photoperiodism, which are generally correlated to these variables, may be influencing phenology (Tauber and Tauber 1976, Nakai and Takeda 1995, Danks

2006). There is no evidence of requirement for diapause between *B. leucopterus hirtus* cohorts. The number of cohorts produced by the related Southern chinch bug, *Blissus insularis* Barber (Hemiptera: Blissidae) varies each year, with a higher number of generations occurring in more southern areas (Potter 1998, Vittum et al. 1999).

The general life cycle *of B. leucopterus hirtus* is as follows: adults of both sexes overwinter in leaf litter, at the base of bunch grasses and other protective locations such as ground mosses or under house siding. In the spring they become active, mate and lay eggs. There are 5 nymphal stages before they become adults (Mailloux and Streu 1981, Potter 1998, Vittum et al. 1999). Both sexes of adult *B. leucopterus hirtus* are dimorphic, exhibiting macropterous (long winged) and brachypterous (short winged) forms. Previous research at sites across their geographic range has shown populations may be univoltine or bivoltine depending on geographic location (Potter 1998). In addition, throughout its range, some oviposition, hatching and early nymphs often appear late in the fall but do not reach the adult stage before winter. However, the lack of nymphs in spring suggests these late season nymphs are unlikely to survive over winter (Leonard 1966).

The geographic expanse of Quebec and Atlantic Canada provides a range of climate conditions, including variation in summer and winter temperature and precipitation, allowing comparison of various life history parameters between locally and regionally separated populations of *B. leucopterus hirtus* under different climate influences. The presence of *B. leucopterus hirtus* across this range, the only subspecies of the *leucopterus* complex found in Newfoundland, presents a unique opportunity to study habitat-induced population variation by using the Southwood habitat templet model as a theoretical foundation, as outlined in *Chapter 1: Introduction*. Historical records show

that the general climatic conditions around St. John's differ considerably from other parts of the insect's range (Figure 1.5). In addition, Newfoundland is an island, and while there is transport to and from the island, there is a presumably lower probability of regional population mixing by natural insect dispersal than between the land-linked provinces of the Canadian mainland. St. John's, NL therefore provides a suitable focal point for this study to explore the variation in phenology of *B. leucopterus hirtus* at a local level and in comparison with other regions.

2.1.3 Rate of Development and Threshold Temperature for Development in *Blissus leucopterus hirtus*

It is well established in the entomological literature that temperature affects insect development rate (Lamb et al. 1984, Nylin and Gotthard 1998) and that differences in development rate may be used as an indicator of inter-population variation in phenology (Traoré et al. 2006). Cool climates generally produce lower development thresholds than warmer climates (Hutchinson and Hogg 1984, Baldwin and Dingle 1986). Because of the cooler summer climate in St. John's, Newfoundland compared to other areas in the Canadian range of *B. leucopterus hirtus*, it is expected that, disregarding any compensatory mechanisms by the insects, development rates and threshold for development would be lower for these populations based on the well-established positive relationship between temperature and metabolism in insects (Nylin and Gotthard 1998).

The threshold for initiation of post-overwintering development for *B. leucopterus hirtus*, as defined by the commencement of oogenesis in females, is 7°C (Liu and McEwen, 1979). Mailloux and Streu (1981) found that the threshold temperature for activity, based on mobility, in *B. leucopterus hirtus* was also 7°C but that the threshold

temperature for egg development was 14.6°C. Based on these results, Wellwood et al. (2002) used a threshold temperature of 7°C to build their model of local phenology in New Brunswick. However, because the applicability of any given threshold temperature across the insect's entire range may vary (Hutchinson and Hogg 1984, Baldwin and Dingle 1986), it is not clear whether either 7°C or 14.6 °C may be an appropriate threshold temperatures on which to compare phenology of populations across a large geographic range with obvious climatic differences.

2.1.4 Phenological Variation in *Blissus leucopterus hirtus*

The geographic range of *B. leucopterus hirtus* creates the potential for variation in phenology among populations at the regional scale based on differences in climate and weather across this range. Evidence from New Brunswick suggests differences occur among populations separated by fewer than 100 km. Variation in phenology among populations separated by only a few kilometers has not formally been assessed, but preliminary observations by the current author suggested it occurred, and lead to testing of this hypthesis in this study. Reference texts on *B. leucopterus hirtus* management suggest pesticide application is most effective when the majority of nymphs are in the 2nd and 3rd instar, when most of the 1st cohort is present and feeding, and most individuals have limited mobility (Johnson 1941, Liu and McEwen 1979, Tashiro 1987, Wellwood et al. 2002). Despite these recommendations, local pest management companies often spray without regard for phenology, or at best, routinely refer to calendar dates when deciding upon spray periods. Given that the time frame in which 2nd and 3rd instars occur depends on the phenology of *B. leucopterus hirtus* at a specific location, understanding this

insect's development in relation to temperature is vital to developing more targeted pesticide location-specific application practices.

Southwood's habitat templet theory predicts that populations optimize life strategy under prevailing environmental conditions. Therefore, differences among phenology at the regional and potentially local scale are expected. Based on the shorter and cooler summer season in St. John's. NL, along with limited population mixing from the mainland, it is expected that the populations in St. John's are univoltine and have a shorter phenology compared to other regions. To achieve a more compact phenology, it is likely that development rate is higher among populations from St. John's, NL than in other regions. Finally, with relation to predictive techniques that can be employed by pest managers, the poikilothermic nature of insects suggests that phenology measured as a function of heat accumulation (cumulative degree days) should be more effective at predicting the appearance of 2^{nd} instars than reference to Julian days.

2.1.5 Research Hypotheses

This study characterized the phenology of *B. leucopterus hirtus* to identify differences in life history among populations at the local and regional scale, for the purpose of better informing pest management plans. Based on current knowledge of *B. leucopterus hirtus* and the habitat template theory, hypotheses are as follows:

1. based on a shorter and cooler summer season compared to other region of the insect's range, populations within St. John's, NL exhibit lower threshold temperatures for egg development and faster rates of egg development;

2. based on environmental differences among sampling location at the local level in St. John's, populations display unique phenologies;

3. based on the shorter and cooler summer season observed in St. John's, NL compared to other regions of the insect's range Newfoundland populations of *B. leucopterus hirtus* are univoltine with a shorter phenology compared to other regions;

4. based on the fact insect that insect development is closely linked with external temperature, Cumulative Degree Days is a more effective means of predicting the appearance of 2nd instars compared to Julian Days.

2.2 Materials and Methods

2.2.1 Egg Development Rate, Determination of a Threshold Development Temperature and Relation to Spatial and Climatic Conditions

2.2.1.1. Collection

In May 2008, overwintered *B. leucopterus hirtus* adults were collected from twelve sites in Quebec and Atlantic Canada. Groups of local populations were sampled within a few kilometers of each other, with the exception of sites in New Brunswick. In New Brunswick, collection was based on local recommendation of sampling sites with two populations sampled in Fredericton, and another in Saint John (separated by 82 km). 'Regional' areas were defined by provincial boundaries, with province names serving as identifiers for groups of local sites. In total, the sampling sites included three in Quebec City (PQ), two in Fredericton (NB), one in Saint John (NB), three in the Annapolis Valley (NS) and three in St. John's, (NL) (Fig. 2.1). Samples were not taken from Prince Edward Island.

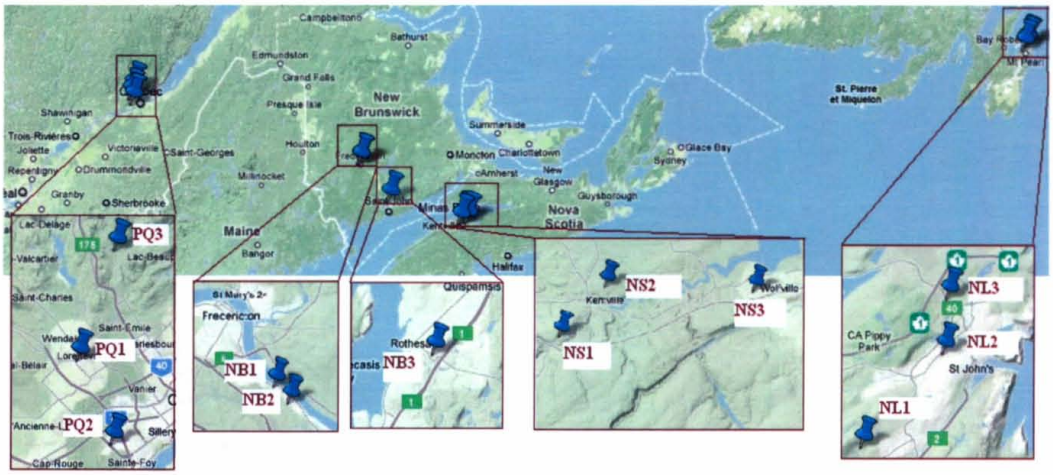

FIGURE 2.1. Location of twelve study sites in Quebec and Atlantic Canada, at each of which *Blissus leucopterus hirtus* were collected over a two week period in May 2008, using a combination of hand sampling and vacuum sampling. Sampling locations were identified by local entomologists as inhabited by *B. leucopterus hirtus* populations.

Individuals were collected over 14 days using a combination of hand sampling and vacuum sampling in areas identified by local entomologists as inhabited by *B. leucopterus hirtus* populations. Sampling methods were chosen for based on speed, efficacy and minimal damage to turf (Appendix C). Captured individuals were placed in plastic containers with grass and plant debris from the local collection area and kept in a container with ice packs that were refrozen daily. Upon arrival in St. John's, the adult bugs were transferred into site-specific large sized plastic *Ziploc*® Snap 'n' Seal Containers, with each container considered an individual colony.

The site specific containers into which the insects were placed had been previously modified by replacing the middle section of the lid with a 0.2 mm mesh that promoted airflow but prevented the bugs from escaping. The size of mesh chosen was based on the minimum 1st instar head capsule width of 0.21 mm recorded by Mailloux

and Streu (1981). Any plant material and debris in the containers was removed and all colonies were fed stem sections from greenhouse grown 3 leaf and 4 leaf stage *Zea mays* var. polka (Veseys Seed Company, Charlottetown, PEI). The colonies were placed into a growth chamber maintained at 20°C±1°C, with a 16:8 hour photoperiod at 100% relative humidity. Although identified as *B. leucopterus hirtus* by the researcher, several individuals were collected from each colony and sent to Agriculture and Agri-Food Canada (AAFC)'s Eastern Cereal and Oilseed Crop Research Centre in Ottawa for official identification by a taxonomic specialist. All samples submitted were confirmed as *B. leucopterus hirtus*. Colonies were then monitored for mating pairs of *B. leucopterus hirtus* adults at 10:00 am each day.

A total of 188 females and their mates were isolated in individual clear plastic 60 mL Solo® snap lid portion cups and labeled with a colony name specific to the province and site of origin (NB1, NB2, NB3, NS1, NS2, NS3, NL1, NL2, NL3, PQ1, PQ2, PQ3). The cups were lined with filter paper and mating pairs were given fresh corn and a moist cotton ball as required. A total of 688 (average eggs per female=3.66) eggs were collected from all mating pairs using a moistened fine artist's paintbrush and were divided as evenly as possible between several plastic cups lined with moistened filter paper. These containers were then distributed among Conviron® growth chambers set at 15°C, 17.5°C, 20°C, 25°C or 30°C with a 16:8 hour photoperiod at 100% relative humidity. Cups were places randomly on trays and trays were rotated within the growth chamber daily. The range of temperatures reflected a spectrum of temperatures experienced across the insect's range during the months of May, June, July and August. The lowest temperature chosen was just above the calculated threshold for oogenesis (14.6°C) derived for a New

Jersey population by Mailloux and Streu (1981) and 30°C was chosen as the highest temperature because it is several degrees lower than the ground temperature recorded by the researcher at several of the collection sites. Egg containers were monitored daily for egg hatch. *Blissus leucopterus hirtus* were considered hatched when more than half of the 1st instar's body had emerged from the egg case. Eggs were allowed to incubate for a maximum of 90 days, after which time unhatched eggs were discarded. An incubation start date for each container was noted and data were recorded as 'days until hatch' for each egg and converted to egg development rate, defined as the reciprocal of the total days until hatch. This gives the proportion of the total egg development time per day. This process was repeated daily until: 1) at least 15 eggs from each site had successfully hatched, or 2) no further eggs were oviposited by females from a given site for 14 days. Mating individuals that did not produce eggs after 14 days were returned to their original colony. Adults that died either during or following the experiment were preserved in 70% ethanol in labeled glass vials for morphometric measurments.

2.2.1.2 Data Analysis

All analyses were performed using JMP® 7.0.1 and JMP® 8.0.2.2 (SAS Institute Inc., Cary, NC, USA) statistical software. Egg development rate was compared among sites using a one-way ANOVA for each temperature. To determine which specific sites differed from one another at each temperature, a post-hoc Tukey test (which corrects for experiment-wide error rate) was performed. A comparison of mean egg development time between geographical regions was achieved by pooling site data from each provincial grouping and performing a one way ANOVA. A Tukey test was again used to identify significant differences between regional pairings.

Following the mathematical procedures of an earlier paper on determining threshold temperature for insect development (Trottier 1971), the threshold temperature for egg development in local and regional populations of *B. leucopterus hirtus* was estimated for each site though iteration with a non-linear model of the hyperbolic equation, $t_i=a(T_i-b)^{-1}$ where T_i is equal to the temperature at which the eggs are reared and t_i is equal to the development time in days. In this equation, the temperature threshold for development is taken from the value of 'b', while 'a' is interpreted as the minimal accumulated temperature in excess of b required for egg development (Trottier 1971, Mailloux and Streu 1981). The threshold temperature for egg development in each region was determined by calculating the mean egg development threshold for three local sites, weighted by the number of eggs hatching at each site.

Canadian climate averages from 1971-2000 were obtained through the Environment Canada website (Environment Canada 2009) for all weather stations in close proximity to sampling locations. In total, this research relied on six weather stations (Annapolis Royal, Jean Lesage International Airport, Fredericton CDA, Saint John, St. John's A, St. John's CDA). Each sampling site was assigned to the closest of the six weather stations and climate data was compared against the weighted mean threshold temperature for egg development of those sites in closest proximity. Using a correlation analysis (both variables continuous), threshold temperature for development at each location was compared with: 1) mean temperature, 2) standard deviation of mean temperature, 3) maximum temperature, 4) minimum temperature, and 5) precipitation for the months of May, June, July and August, periods during which 1st cohort eggs may be present in one or more site locations.

2.2.2 Modeling of General Phenology for *Blissus leucopterus hirtus* at a Local Level

2.2.2.1 Collection

In 2004 and 2005, ten sampling sites in St. John's, NL were selected based on reports of high infestation gathered though personal correspondence and an appeal for study properties during a radio interview hosted by the Canadian Broadcasting Corporation. Four residential properties, four sites in St. John's city parks and two sites at AAFC's Cool Climate Crop Research Centre were selected (Fig. 2.2). The relative proximity of some sites reflects opportunistic sampling in areas where *B. leucopterus hirtus* were present and the accessibility of sites in the city.

FIGURE 2.2. Location of ten local study sites within St. John's, Newfoundland and Labrador. Sites are designated with an identifying letter. Due to the destruction of site C1 in late 2004, C1 was replaced by C2, a site with similar characteristics, in 2005. Sampling was conducted at approximately one week intervals for 18 weeks per year from early June until late October in 2004 and 2005. At the onset of the project in 2004, delays in locating sites of low density *B. leucopterus hirtus* prevented a full 18 week sampling regime at some sites. In 2005, all sites were sampled 18 times.

Sampling was conducted at approximately one week intervals for 18 weeks per year from early June until late October in 2004 and 2005. At the onset of the project in 2004, delays in locating sites of low density *B. leucopterus hirtus* prevented a full 18 week sampling regime at some sites. In 2005, all sites were sampled 18 times. Based on personal observations, *B. leucopterus hirtus* appeared to be most active and visible under sunny conditions and warm temperatures. Therefore, whenever possible, sampling was conducted on warm, sunny days.

A plot of approximately 2 m^2 was selected for sampling at each site in the spring of 2004. The sampling process initiated each week with a visual search of the plot to determine the location of highest density of *B. leucopterus hirtus*. Floatation sampling was used to collect the insects. The decision to use this method was based on an experimental comparison of several sampling methods as part of the current study (Appendix C) and in consideration of the need to compare results across studies that also used the floatation method. At each sampling site, a PVC pipe (d=15 cm and h=20 cm) with a beveled edge was inserted approximately 2.5 cm into the ground using a mallet. The cylinder was then filled ¾ full with water. *Blissus leucopterus hirtus* that floated to the surface were collected using a fine mesh tea strainer until no additional specimens were seen on the surface of the water and placed in 500 mL plastic containers labeled with date, time and location. The sample was collected over a ten minute period with water replenished as required. The turf within the cylinder was agitated using a knife at 2, 5 and 8 min. Samples were taken to the laboratory and frozen for later assessment. After thawing, *B. leucopterus hirtus* were removed from the debris with the aid of a magnifier light, tweezers and a fine artist's paintbrush. Specimens were placed on filter paper in

Petri dishes and identified to life stage using a stereomicroscope. Adult sex and wing form were also recorded. All specimens were then preserved in sealed glass vials containing 70 % ethanol and labeled with pertinent sample information.

2.2.2.2. Data Analysis

To compensate for potential differences in individual counts on sampling days due to differences in sampling size among sites and between years, sample counts of each instar were interpreted as proportions of the total sample. The day of maximum proportion for a particular instar was defined as either the Julian date or cumulative degree day (CDD) on which that instar represented the highest proportion of the total sample compared to its contribution on all other sampling days. This date was determined through visual examination of the data. Hourly air temperature data obtained from the AAFC Atlantic Cool Climate Crop Research Centre weather station were used to convert sampling dates to cumulative degree days (CDD) from January 1st in each year, using both a 7.00°C threshold temperature and the calculated egg development threshold temperature of 15.42°C derived from the methods described in section 2.2.1.2.

All analyses were performed using JMP® 7.0.1 and JMP® 8.0.2.2 (SAS Institute Inc., Cary, NC, USA) statistical software. Graphs of phenology based on Julian days and CDD were created in MiniTab 14®. Inter-site variation was examined using all three phenology descriptors (Julian days and CDD based on 7.00°C and 15.42°C threshold temperature). 'Day of maximum proportion' from each site in 2004 and 2005 were compared for each instar using a one-way ANOVA test with Bonferroni correction. To identify inter-year variation in 'day of maximum proportion', pooled site data were

compared between 2004 and 2005 for each instar using a matched pair analysis with Bonferroni correction. Adult insects were omitted from these comparisons because the maximum occurrence of this stage often fell on the first or last sampling day, which varied between sites in some years. This would have therefore skewed a comparison of 'day of maximum proportion' for adults based on Julian date or CDD. The phenology models developed for St. John's, NL for 2004 and 2005 were converted to graphical charts and visually compared with phenologies determined through previous studies in Fredericton, NB, Canada and Metuchen, NJ, USA.

2.3 Results

2.3.1 Comparison of Egg Development, Threshold Temperature for Egg Development and Relation to Spatial and Climatic Conditions

2.3.1.1 Rate of Egg Development

Of the total 688 eggs incubated, 550 hatched (82%). There was no egg hatch in 90 days at either 15.0°C or 17.5°C. During that time period, the majority of the eggs reared at 17.5°C commenced development and turned a pinkish colour, but then apparently ceased development and shriveled. Eggs reared at 15.0°C and 17.5°C were, therefore, eliminated from analyses. Egg development rate is defined as the reciprocal of the total days until hatch and reflects the proportion of total egg development until hatch occurring on each day. Egg development rate increased with temperature at each of the 12 sites. The development rate differed significantly among all twelve sites at all three experimental temperatures (20°C: $F(11, 170) = 10.54$, $p<0.0001$, 25°C: $F(11, 163)=4.04$, $p<0.0001$,

30°C: F(11, 181), p <0.0001) Post-hoc analysis included using a Tukey test to determine which pairs of means were significantly different (Table 2.1).

TABLE 2.1. Mean rate of egg development with Standard Error (SE) in *B. leucopterus hirtus* at three temperatures for 12 sites in Quebec and Atlantic Canada. Sites are listed by decreasing mean for each development rate. Means that share an alphabetical notation listed to the immediate right of the SE are not significantly different based on post-hoc Tukey test analysis at p=0.05.

Temperature											
20°C				***25°C***				***30°C***			
Site	**Mean Rate of egg development (% per day)**	**SE**		**Site**	**Mean Rate of egg development (% per day)**	**SE**		**Site**	**Mean Rate of egg development (% per day)**	**SE**	
NL2	3.59	0.0504	A	NL3	7.69	0.1108	AB	NL3	12.50	0.1563	A
NL3	3.59	0.0401	A	NL1	7.65	0.0946	A	NL1	11.92	0.1344	A
NL1	3.58	0.0451	A	NS3	7.42	0.0969	AB	NL2	11.43	0.1478	AB
NS2	3.50	0.0527	AB	NL2	7.31	0.1153	AB	NS2	11.04	0.1860	BC
PQ1	3.43	0.0324	ABC	NS2	7.16	0.0729	ABC	NB1	11.00	0.1157	BC
NS3	3.32	0.0582	BC	NB2	7.13	0.1997	BC	PQ2	10.90	0.3720	BCD
NB1	3.32	0.0504	BC	NB3	7.12	0.0969	BC	NB3	10.76	0.1664	CD
NS1	3.31	0.0372	BC	NS1	7.11	0.0999	BC	PQ3	10.69	0.1664	CD
NB3	3.30	0.0451	C	PQ1	7.08	0.1068	ABC	NB2	10.69	0.1519	CD
PQ3	3.26	0.0504	BC	NB1	7.04	0.1331	BC	NS3	10.49	0.1664	CD
PQ2	3.24	0.0401	C	PQ3	7.03	0.1108	BC	PQ1	10.46	0.1787	CD
NB2	3.23	0.0660	C	PQ2	6.86	0.1153	C	NS1	10.24	0.1860	D

For purposes of regional contrast, mean development rate of each provincial grouping was compared. There was a significant difference between mean provincial egg development rates at 20°C: F(3, 178)=27.69, p<0.0001, 25°C: F(3, 171)=9.29, p<0.0001 and 30°C: F(3, 189)=23.16, p<0.0001 (Fig. 2.3). A post-hoc Tukey test showed the

Newfoundland grouping to have a significantly higher development rate from at least one other regional grouping at all three experimental temperatures (Fig. 2.3).

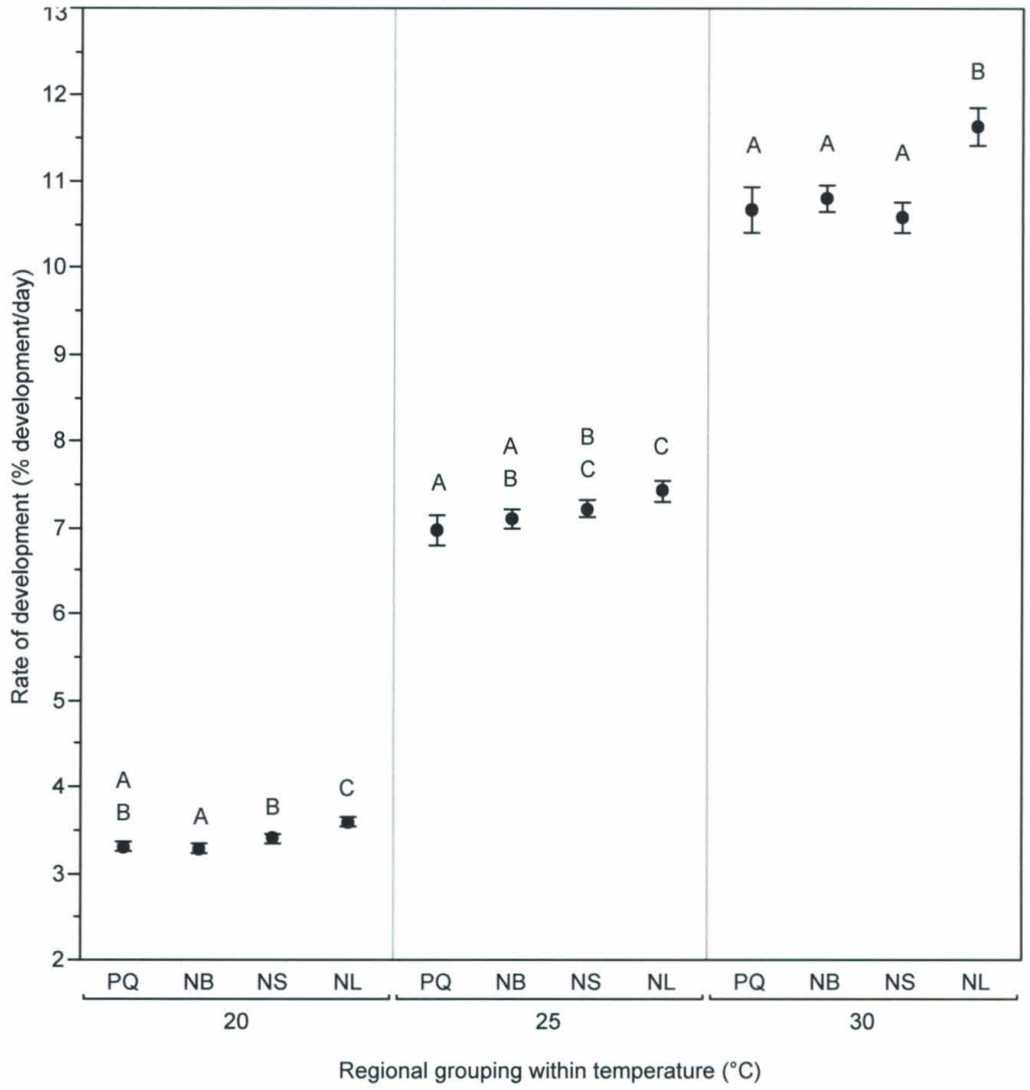

FIGURE 2.3. Mean rate of development of *B. leucopterus hirtus* at three temperatures for four regional groupings in Quebec and Atlantic Canada. Confidence ranges of 0.95 are shown. Means that share an alphabetical notation are not significantly different based on post-hoc Tukey test analysis at p=0.05.

2.3.1.2 Threshold Temperature for Egg Development

Iteration of the hyperbolic function $t_i=a\ (T_i-b)^{\wedge -1}$ produced slightly different estimates of threshold temperature for egg development among sites, with the threshold varying between 15.18°C and 15.76°C. Weighted means of provincially grouped sites again revealed similar threshold temperatures required for egg development, ranging from 15.42°C to 15.56°C (Table 2.2). A one way ANOVA determined that there was no significant difference in mean threshold temperature between regional groupings ($p=0.9363$).

TABLE 2.2. Estimated threshold temperature for rate of egg development among individual sites, with weighted mean threshold temperature for regional groupings.

Site	n	Estimated Threshold Temperature (°C)	Approx. Standard Error	Regional Grouping	Estimated Threshold Temperature (°C) (Weighted Mean)
PQ 1	**36**	15.18	0.1926	**PQ**	15.49
PQ 2	**45**	15.62	0.1381		
PQ 3	**51**	15.60	0.1664		
NB 1	**42**	15.60	0.1251	**NB**	15.56
NB 2	**60**	15.77	0.1336		
NB 3	**51**	15.27	0.1066		
NS 1	**44**	15.61	0.1607	**NS**	15.50
NS 2	**53**	15.28	0.1158		
NS 3	**47**	15.66	0.1242		
NL 1	**35**	15.68	0.1830	**NL**	15.42
NL 2	**90**	15.28	0.1213		
NL 3	**16**	15.65	0.1277		

2.3.1.3 Relation of threshold temperature for egg development to environmental conditions

There was no relationship between threshold temperature for egg development and 1) average monthly temperature standard deviation of average monthly temperature, 3) average maximum monthly temperature and 4) average minimum monthly temperature or 5) precipitation from nearby weather stations for the months May through August (Table 2.3).

TABLE 2.3. Summary statistics (r^2, p-value and Least Significant Number (# of data points required for significance at p=0.05)) for correlation between threshold temperature for egg development in *B. leucopterus hirtus* and A) average monthly temperature (°C) B) standard deviation of average monthly temperature (°C), C) average monthly maximum temperature (°C), D) average monthly minimum temperature (°C), E) average monthly precipitation (mm) collected from 6 weather stations in the insect's range. The value for threshold temperature for egg development is based on the weighted mean from the collection sites in closest proximity to each weather station.

Climate Variable	**Month**			
	May	June	July	August
Mean Average Temperature	r^2=0.0001 p=0.9877 LSN=85761	r^2=0.0042 p=0.9027 LSN=1363	r^2=0.0166 p=0.8087 LSN=344	r^2=0.0130 p=0.8300 LSN=442
Mean Standard Deviation of Average Temperature	r^2=0.1847 p=0.3950 LSN=28	r^2=0.1967 p=0.3783 LSN=26	r^2=0.2563 p=0.3055 LSN=50	r^2=0.0173 p=0.8037 LSN=19
Mean Maximum Temperature	r^2=0.0001 p=0.9871 LSN=77327	r^2=0.0007 p=0.9593 LSN=7824	r^2=0.0087 p=0.8603 LSN=658	r^2=0.0011 p=0.9512 LSN=5437
Mean Minimum Temperature	r^2=0.0053 p=0.8915 LSN=1094	r^2=0.0111 p=0.8429 LSN=518	r^2=0.0423 p=0.6959 LSN=133	r^2=0.0549 p=0.6550 LSN=102
Mean Precipitation	r^2=0.2118 p=0.3585 LSN=24	r^2=0.0117 p=0.8382 LSN=488	r^2=0.0555 p=0.6532 LSN=101	r^2=0.0008 p=0.9574 LSN=7133

2.3.2 Modeling the Phenology of *Blissus leucopterus hirtus* at a Local Level

2.3.2.1 Phenological Variation Among Local Sites in St. John's, NL

In the St. John's, Newfoundland and Labrador region, 6008 specimens from 138 samples in 2004, and 3703 bugs from 180 samples in 2005 were collected. It was noted in the field that conspicuous populations and associated damage by *B. leucopterus hirtus* were less evident in 2005 than 2004. Data from the two sampling years showed only one completed cohort of *B. leucopterus hirtus* in each year within St. John's. Although some

oviposition and egg hatch occurred in late summer, there was no evidence that individuals from the 2nd cohort completed development beyond the second instar prior to winter.

Cumulative degree days were calculated using both the threshold temperature of 7°C employed in previous studies (Liu and McEwen 1979, Wellwood et al. 2002) and the egg development threshold of 15.42°C for St. John's, NL as determined in the current study. Because the objective was to compare the egg development threshold as a baseline temperature in phenology in St. John's, NL, the New Jersey based egg development threshold of 14.6°C by Mailloux and Streu (1981) was not included in comparison. The threshold determined through this study in St. John's using the same methods was assumed to be more reflective of local populations. The temporal occurrence of all life history stages showed a similarity in the pattern of development between 2004 and 2005 based on pooled site data from St. John's using Julian days and degree-days with a threshold of 7°C and 15.42°C (Figs. 2.4, 2.5, 2.6).

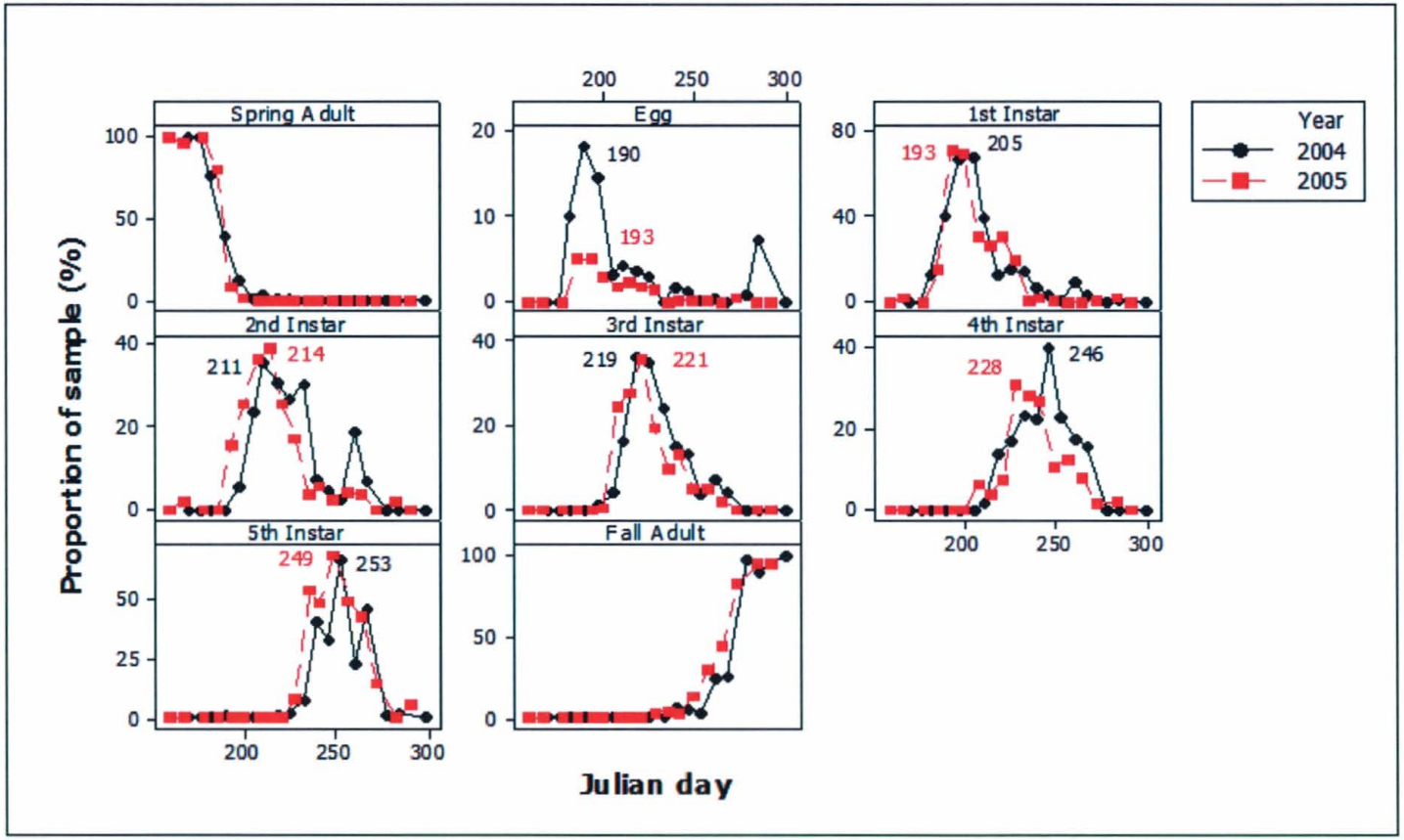

FIGURE 2.4. Proportion of total sample comprised of individuals in each life stage from samples collected in 2004 and 2005. The inflection point (in Julian days) is indicated by the colour coded value beside each peak.

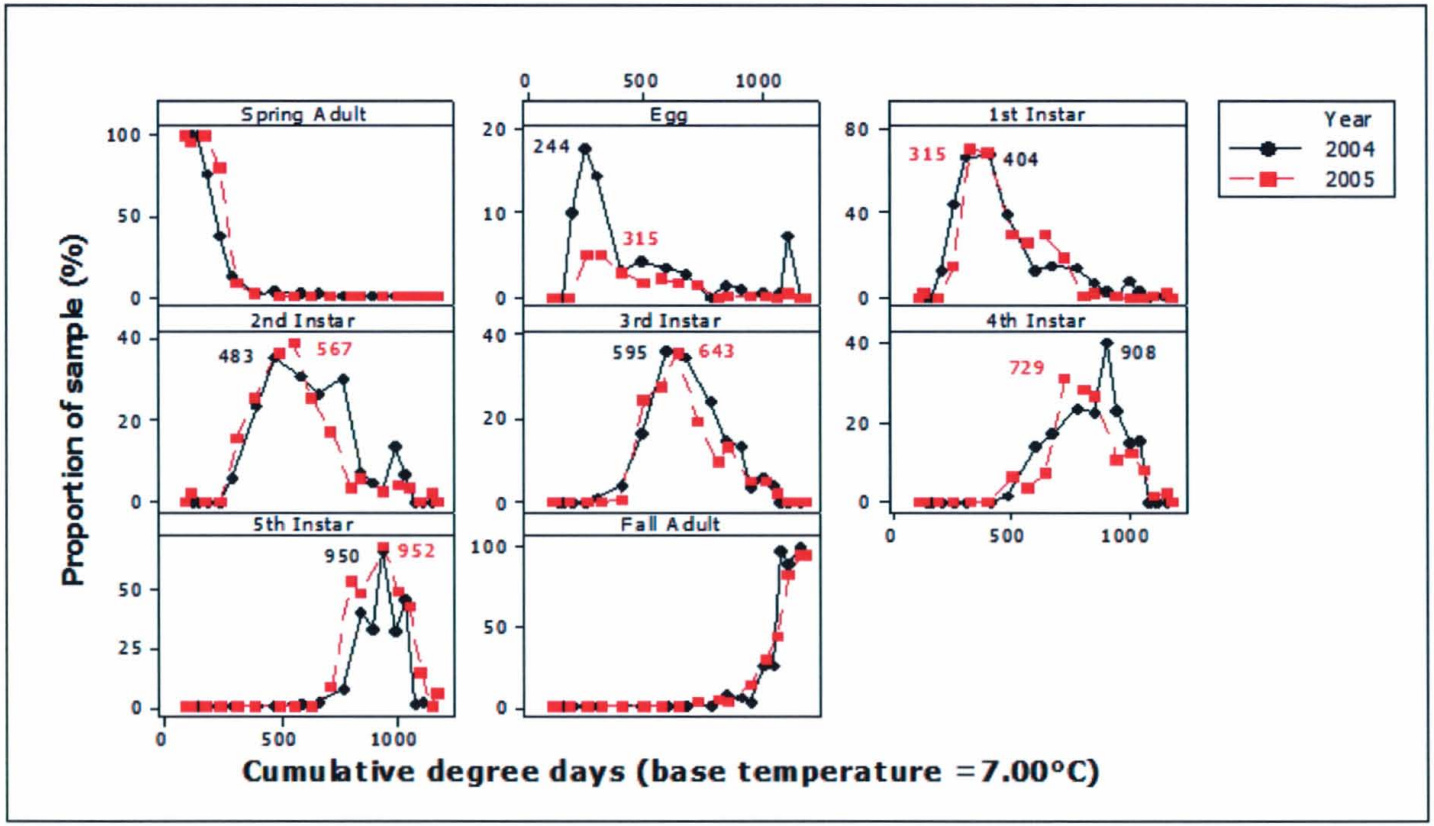

FIGURE 2.5. Proportion of total sample comprised of individuals in each life stage from samples collected in 2004 and 2005. Sampling days are represented by the equivalent number of CDD on that sampling day, calculated using a threshold temperature of 7.00°C. The 'day maximum proportion' is indicated by the colour coded value beside each peak.

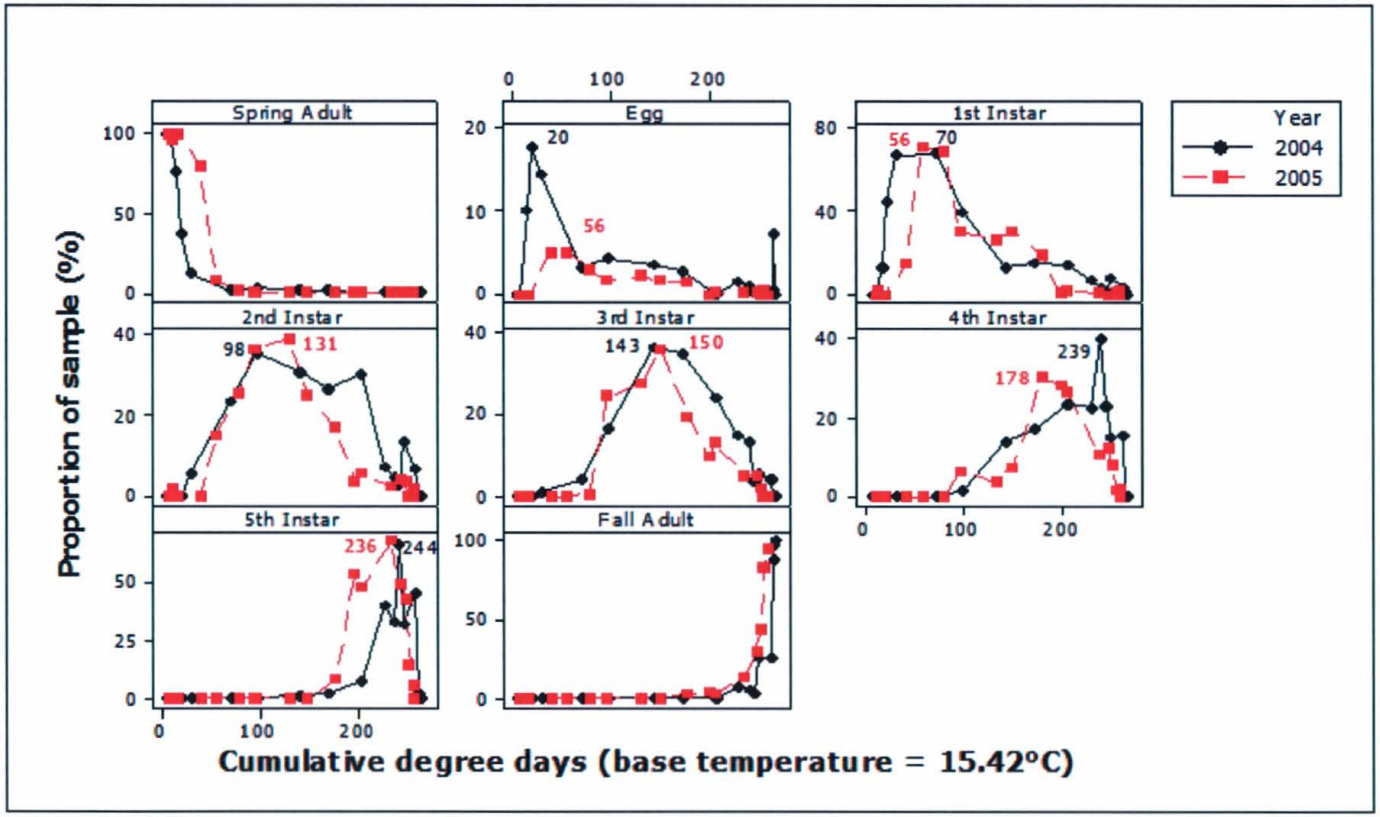

FIGURE 2.6. Proportion of total sample comprised of individuals in each life stage from samples collected in 2004 and 2005. Sampling days are represented by the equivalent number of cumulative degree days on that sampling day, calculated using a threshold temperature of 15.42°C. The 'day of maximum proportion' is indicated by the colour coded value beside each peak.

An inter-site comparison in St. John's, Newfoundland and Labrador, using three measures of phenology (Julian days and CDD using 7.00°C and 15.42 °C) revealed no difference in the 'day of maximum proportion' point for most life stages among sites, except for occurrence of first instars using both methods of CDD (Table 2.4). Because of the multiple paired test performed for each measurement type, there significance level has been adjusted using the Bonferroni correction, from p =0.05 to p=(0.05/6)=0.0084. The site data was pooled the data to facilitate further comparisons among years of study.

TABLE 2.4. p-values generated from a series of one way ANOVAs comparing 'day of maximum proportion' for eggs and instars between ten local St. John's sites, using three measures of chronology. Results were not significant at p=0.0084.

X variable		**Instar**				
	Egg	*1st instar*	*2nd Instar*	*3rd Instar*	*4th Instar*	*5th Instar*
Julian day	0.7110	0.1696	0.9218	0.2033	0.7002	0.4243
CDD using threshold temperature of 7.00°C	0.7179	0.0478	0.8826	0.1812	0.5930	0.3076
CDD using threshold temperature of 15.42 °C	0.7327	0.0484	0.8626	0.3124	0.6831	0.7024

The 'day of maximum proportion' comparison between 2004 and 2005 (Table 2.5) supports the visual representation of the phenology (Fig. 2.4, 2.5, 2.6) showing similar patterns in phenology between years in most instars. Because of the multiple paired tests performed here, there significance level has been adjusted from p =0.05 to

p=(0.05/6)=0.0084). Therefore, the 'day of maximum proportion' for both 1st and 2nd instar nymphs, which would normally be significant different at p=0.05 between years when the phenological descriptor of Julian days was used, cannot be considered significant here. The same applies for 4th and 5th instar nymphs between years when the phenological descriptor of CCD with a threshold temperature of 15.42°C was used.

TABLE 2.5. p-values for comparison of 'day of maximum proportion' between 2004 and 2005 for each instar of *B. leucopterus hirtus* using three units of measurement for 'day of maximum proportion' at the St. John's, Newfoundland and Labrador sites. Results were not significant at p=0.0084.

X variable	**Instar**					
	Egg	*1st instar*	*2nd Instar*	*3rd Instar*	*4th Instar*	*5th Instar*
Julian day	0.972	0.0259	0.0485	0.3059	0.1566	0.1868
CDD using threshold temperature of 7.00°C	0.7219	0.3899	0.1540	0.5096	0.2235	0.3469
CDD using threshold temperature of 15.42 °C	0.6575	0.7393	0.1012	0.2434	0.0478	0.0123

2.3.2.2. Phenological Variation Between Regions in Atlantic Canada and the Northeastern United States.

Using the available phenology data from previous studies, the occurrence of adults and nymphs within various populations throughout the range was compared (Fig. 2.8). The 1976 data from New Jersey showed a bivoltine population with no initiation of a third cohort within the year. The study from Fredericton, NB revealed a univoltine population with initiation of a second cohort that did not develop completely before the

winter season. Populations in St. John's also demonstrated a univoltine population, with later initiation of a second cohort, and detection of only 1st instar before winter in 2004 and 2nd instars in 2005.

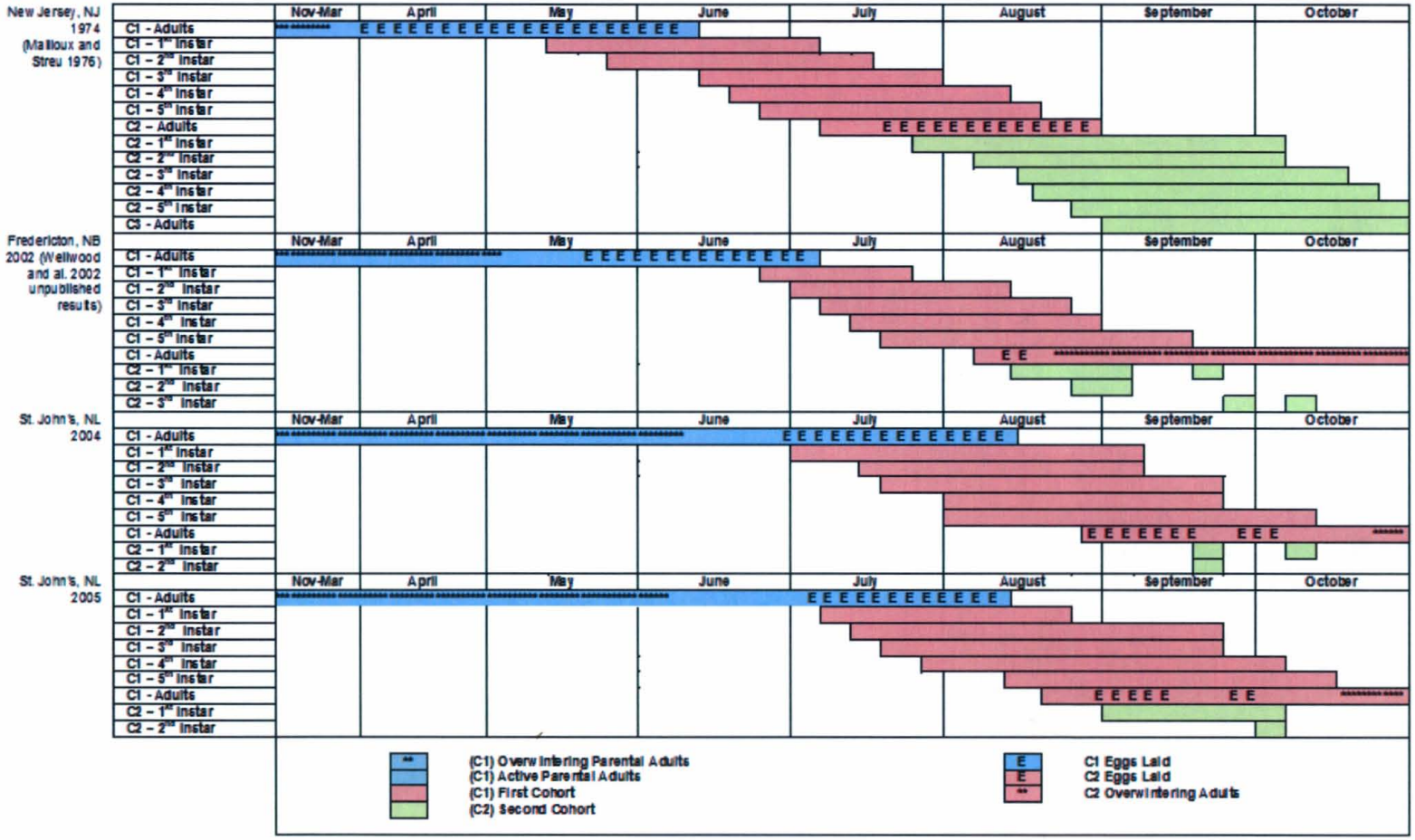

FIGURE 2.7. Comparison of phenology for *Blissus leucopterus hirtus* using data obtained from two previous phenology studies and data obtained through this study in 2004 and 2005. Adapted from Wellwood et al. (2002).

2.4 Discussion

2.4.1 Egg Development Rate in *Blissus leucopterus hirtus*

The results in this study concur with other studies suggesting that temperature is an important abiotic influence in life history. Within this study, the variation in egg development rate was greater among geographical regions than between individual sites within the same province. At each of the three incubation temperatures, the NL regional grouping had the highest development rate and was significantly different from at least one other provincial grouping. At both 20°C and 25°C, at least two other regional groupings of sites also exhibited significant differences amongst one another, suggesting that at lower development temperatures, the variation in development rate among regional groups was either greater than at higher temperatures or more easily detected at lower temperatures due to greater relative precision in development times resulting from longer incubation times.

The faster rate of egg development time in St. John's in *B. leucopterus hirtus* was particularly evident at 30°C, a temperature at which the other regions had a non-detectable difference in development rate among them. These faster development times aligns with other examples cited by Denno and Dingle (1981). For example, the migration, diapause and life history characteristics of *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae), were shown to have adapted to regionally distinct climate conditions in Iowa, Puerto Rico and Florida (Dingle 1981). The faster development time in *B. leucopterus hirtus* also supports another regionally based study in which ecotypes of

Anaphes victus Huber (Hymenoptera: Mymaridae) in cooler climates of Michigan and Quebec were found to have faster base development rates than populations in relatively warmer Texas, where mean temperatures are higher (Traoré et al. 2006). The higher base development rate may result in optimized growth by *B. leucopterus hirtus* in St. John's during intermittent periods of warmer weather. Based on the regional constraints of temperature, evidence suggests *B. leucopterus hirtus* has responded to regionally variable conditions across its Eastern range. As predicted, populations within St. John's, NL have exhibited faster rates of egg development compared to other regions of the insect's range. These results confirm the need for regional specificity in life history characterization of this species, and add regional context to life history studies conducted in other parts of the insect's range. For example, predictions of infestation based on threshold temperatures and development rates of nymphal instars in Ohio (Niemczyk et al. 1992), should be considered in a regional context. For pest managers, they should be aware that *B. leucopterus hirtus* may respond to favourable conditions in St. John's, NL through development faster than they would in other regions.

A comparison of threshold temperatures of egg development rate outside of the current study area of Quebec and Atlantic Canada provided additional insight into the regional life history adaptations of this species. In this study, an incubation temperature of 30°C resulted in egg development rates ranging from 10.24%-12.50% per day, which are equal to, or lower, than those found in the biovoltine population of New Jersey, where the egg development rate at 30°C was an average of 12.50 % per day (Mailloux and Streu 1981). This result appears at first to contrast a simplistic model in which development

rate decreases within transition to warmer and longer growing seasons. However, the ecological opportunity to complete two generations per year may be a selection pressure for faster development rates in the climate of New Jersey compared to northern regions. While populations of *B. leucopterus hirtus* in St. John's have apparently been selected for lower threshold temperature for egg development and faster development rate compared to populations in slightly warmer Canadian regions, populations in New Jersey have seemingly adapted increased developmental rates to accommodate *two* cohorts per year within the slightly longer favourable season.

This type of pattern, known as saw tooth variation, has previously been described as an explanation for oscillating development times (Roff 1980, Bradford and Roff 1995) and has been documented in crickets (Masaki 1978) and butterflies (Nylin and Svärd 1991). This pattern develops as a result of insects attempting to fit a discrete number of cohorts into a single growing season. If the number of cohorts per year (n) is possible at a given location, then at each geographical interface at which a greater number of cohorts may be possible, an insect may adapt a shorter life cycle in order to accommodate (n+1) cohorts per year. As the severity and duration of winter decreases, most often with latitude (Danks 2006), an increasingly longer growing season would permit greater development time for each cohort until conditions make it possible to accommodate an additional cohort and the pattern is repeated. The implication that *B. leucopterus hirtus* may exhibit a saw tooth cline pattern is important for predictive climate-insect models, as recent work has confirmed the potential for climate induced increases in multivoltism in northern regions (Pöyry et al. 2011). If temperature regimes do shift over time, it is

possible that the range of *B. leucopterus hirtus* may shift north and that the number of cohorts per year may increase.

2.4.2 Threshold Temperature for Egg Development in *Blissus leucopterus hirtus*

Based on Trottier's iterative formula that used egg development rate to predict threshold temperature for egg development, St. John's, NL had the lowest threshold temperature compared to other locations in this study. The fact that the threshold temperature across regions differed by less than one degree centigrade fits well with the finding by Morris (1971) that only small differences exist in development threshold across provinces. These results present patterns that agree with previous research suggesting that the cooler climates associated with higher latitudes tend to result in lower thresholds for development than do warmer climates (Campbell et al. 1974, Trimble and Lund 1983, Hutchinson and Hogg 1984, Baldwin and Dingle 1986). For example, populations of both *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae) and *Brevicoryne brassicae* L. (Hemiptera: Aphididae) in Vancouver, Canada had lower threshold temperatures than those populations in Berkley, California, USA, (a region with comparatively higher mean temperatures than Vancouver), but appeared to compensate for this fact by having a higher intrinsic rate of development (Campbell et al. 1974). Campbell et el. (1974) also suspected that development of threshold temperatures was most strongly influenced by variability in temperature at the beginning of the season. Unfortunately, within this study, there were no significant correlations between threshold temperature for egg development and historic mean average temperature, mean standard

deviation, mean maximum temperature, mean minimum temperature nor precipitation from closely located weather stations.

The threshold temperature for egg development calculated in New Jersey, using Trottier's hyperbolic equation (Trottier 1971), was 14.6°C (Mailloux and Streu 1981), while threshold temperatures derived in St. John's, NL were a full degree higher. The results from this study do not fit the generality that threshold temperature for development increases with increased regional temperatures (Campbell et al. 1974). However, this lower threshold for development may be a reflection of suspected saw tooth cline in life cycle characteristics as discussed previously.

Although the threshold temperatures for egg development were calculated as 14.6°C in NJ and between 15.18°C – 15.77°C among populations in this study, eggs reared at 15°C and 17.5°C failed to fully develop and hatch. Perhaps temperatures in this range may allow for initiation of egg development but periodic or sustained higher temperatures may be required for complete development to hatching. Furthermore, although the threshold temperature for egg development in St. John's, NL was calculated at 15.42°C, there were very few days on which the average air temperature recorded from meteorological stations exceeded this temperature. Possible explanations for development despite these lower recorded temperatures may include capitalization on short periods of high temperature or warmer oviposition sites selected by females. These sites could include tiller bases and mosses near the soil, which are able to provide a warmer microclimate than the ambient air temperature. Examples of this have been recorded in other insect species, including tiger swallowtail butterflies *Papilo glaucus* L. (Hemiptera:

Papilionidae). The laying of eggs by this insect in warmer microclimates (in direct sun exposure) has altered phenology by up to 11 days (Grossmueller and Lederhouse 1985). It is likely that the temperature experienced by *B. leucopterus hirtus* eggs during development is higher than the mean air temperature during at least some time periods. Although low-interval site specific soil surface temperature measurements were not rigorously collected during this study, occasional soil surface measurements using an infrared thermometer revealed ground temperatures well above 30°C at many study sites. Future work may find soil temperatures provide increased accuracy in predicting phenology, and specificity in hatching time.

2.4.3 Modeling the Phenology of *B. leucopterus hirtus*

Overall, this study does not support phenological adaption in *B. leucopterus hirtus* at the local scale within St. John's, NL. It is possible that weekly sampling was simply not adequate to detect phenological differences among local sites that may be defined by only several days. The phenological patterns for 2004 and 2005 were similar when calculated as a function of JD and CDD based on both 7.00°C and 15.42°C baseline temperatures. The graphical curves representing 2004 and 2005 were much more closely aligned for models using JD and CDD 7.00°C, which is most likely due to greater precision in JD and accumulated temperatures using a lower base temperature compared to the higher base temperature of 15.42°C. It is likely that *B. leucopterus hirtus* is still developing physiologically during the period when ambient air temperatures are less than 15.42°C (but microclimates are warmer) and so that growth is better captured using a lower base air temperature. The similar phenology among insects at local sites in St.

John's suggests that either differences in land use, maintenance level and initial density of *B. leucopterus hirtus* at the site have little impact on the timing of development at this local scale or that sites sampled likely possess a similar suite of environmental characteristics that produce similar population dynamics.

Using the 7°C threshold temperature for measuring phenological development, this study found that the St. John's populations required a lower CDD to reach key developmental stages compared to those in warmer or more southern regions. The cumulative degree-day period during which 2nd and 3rd instars were present corresponds to 281 CDD-623 CDD in 2004, and 298 CDD-670 CDD in 2005. Results from New Brunswick report 2nd and 3rd instars between 423 CDD -877 CDD (Wellwood et al. 2002). In Ontario, which also experiences one generation of *B. leucopterus hirtus* per year, third instars peaked at 750-950 CDD (Liu and McEwen 1979), and, in separate observations, at 850 DD (Sears et al. 1980).

There are notable differences between life history strategies in St. John's and Fredericton, NB. Both spring oviposition and the initiation of a second cohort occur later in the season in St. John's compared to Fredericton and other locations in New Brunswick. In general, *Blissus leucopterus hirtus* within St. John's, NL have adapted a univoltine strategy with a high rate of egg development compared to other regions across the Eastern Canadian range. The differences in regional phenology identified through comparison among multiple studies suggest that seasonal development is affected by larger scale regional and temporal differences in climate and weather patterns that

typically vary among years and over large geographical areas (Figure 1.5). These finding indicate that life history adaptation has occurred within populations of *B. leucopterus hirtus* in response to unique climate templets across Quebec and Atlantic Canada. More specifically, the results support the case for evolutionary adaptation or placticity of this species at a regional scale, and are therefore in line with the prediction that habitat variability produces marked differences in life history (Southwood 1977). Although the populations in St. John's, NL and other regions can be identified as primarily univoltine or bivoltine, there is evidence in these studies that there is initiation of an additional cohort towards the end of the seasonally optimal growth period. Because no eggs or young instars were found in the spring of the following year, it is assumed that these eggs and young instars did not survive the winter. The initiation, but non-completion of a second or third cohort is common among temperate insects, as they attempt to accommodate additional cohorts in a short growing season (Wiklund et al. 1992). An explanation for this increased energy cost and mortality may be explained by bet-hedging. Using this ecological strategy, insects adapt within the context of an unpredictable environment to spread risk over a period of time (Sota 1988, Danks 2007). In the case of *B. leucopterus hirtus*, this strategy has allowed the species to develop a multivoltine life history that maximizes the number of cohorts per year in a seasonal and unpredictable environment. A similar example is found in *Lygaeus equestris* L. (Heteroptera: Lygaeidae), a species which, although once considered "univoltine" was found to have a at least a partial second generation during particularly warm and/or otherwise favourable years (Solbreck and Sillén-Tullberg 1981).

Julian days were as effective as cumulative degree days predicting occurrence of various instars in 2004 and 2005. This suggests that there are drivers other than temperature, such as genetics or photoperiod (Beck 1980, Danks 2007) that are influencing development. Further research would be necessary to determine the factors that most closely influence and/or predict physiological development of this insect. The fact that the Julian days associated with instar appearance were very similar in 2004 and 2005 suggests that pest managers may be able to use this simple system to predict appropriate spray intervals.

2.5 Summary

As predicted, the cooler and shorter summer season in St. John's, NL has produced a lower threshold temperature for egg development and a faster egg development rate. The variability of the response of *B. leucopterus hirtus* to unique environmental conditions is consistent with the habitat templet model (Campbell et al. 1974, Southwood 1977, Solbreck 1978). In St. John's, NL, there was minimal difference between phenology at 10 sites in the city, providing little evidence of ecological adaptation at the local level in response to habitat conditions. The prediction of a univoltine population was correct, and when pooled data from the 10 sites in St. John's were compared with data across larger geographical regions, differences in peak instar occurrence were found, suggesting variation in phenology at the regional scale. Modifications may be required for the development of effective pest mitigation for *B. leucopterus hirtus* in St. John's, NL as this study demonstrates the need to incorporate

local specificity of insect development rate. Differences in regional phenology confirm the need for vigilance because of the potential of *B. leucopterus hirtus* to thrive in distinct habitats. If temperature regimes do shift over time, it is possible that the range of *B. leucopterus hirtus* may shift north and that the number of cohorts per year may increase. Contrary to the initial hypothesis, comparison of methods for measuring phenology (JD, CDD 7.00°C and CDD 15.42°C) in *B. leucopterus hirtus* provided similar results in predicting peak occurrence of instars. However, these results mean the pest managers have use of severable options for predicting appearance of key instars. It is clear from the results of Chapter 2 that environmental conditions have influenced the life history of *B. leucopterus hirtus*, resulting in specific adaptations to optimize life strategy in distinct climates.

Literature Cited

Baldwin, J. D., and H. Dingle. 1986. Geographic variation in the effects of temperature on life history traits in the large milkweed bug *Oncopeltus fasciatus*. Oecologia (Berlin) 69:64-71.

Beck, S. D. 1980. Insect Photoperiodism, 2nd ed. Academic Press, New York, New York, USA.

Bradford, M. J., and D. A. Roff. 1995. Genetic and phenotypic sources of life history variation along a cline in voltinism in the cricket A*llonemobius socius*. Oecologia 103:319-326.

Brown, W. J. 1949. Collected Specimen. 1 female, St. John's, 16.VII. Canadian National Collection, Ottawa, Ontario, Canada.

Campbell, A., B. D. Frazer, N. Gilbert, A. P. Gutierrez, and M. Mackauer. 1974. Temperature requirements of some aphids and their parasites. Journal of Applied Ecology 11:431-438.

Danks, H. V. 1994. Diversity and integration of insect control in insects. Pages 5-40 *in* H.V. Danks, editor. Insect life-cycle polymorphism: theory, evolution and ecological consequences for seasonality and diapause control. Kluwer Academic Publishers, Dordrecht, Germany.

Danks, H. V. 2006. Key themes in the study of seasonal adaptations in Insects II. Life-cycle patterns. Applied Entomology and Zoology 41:1-13.

Danks, H. V. 2007. The elements of seasonal adaptations in insects. Canadian Entomologist 139:1-44.

de Block, M., and R. Stoks. 2004. Life history variation in relation to time constraints in a damselfly. Oecologia 140:68-75.

Demont, M., and W. U. Blanckenhorn. 2008. Genetic differentiation in diapause response along a latitudinal cline in European yellow dung fly populations. Ecological Entomology 33:197-2001.

Denno, R. F., and H. Dingle. 1981. Considerations for the development of a more general life history theory. Pages 1-6 *in* R. F. Denno and H. Dingle, editors. Insect life history patterns: habitat and geographic variation. Springer-Verlag, New York New York, USA.

Dingle, H. 1981. Geographic variation and behavioural flexibility in the milkweed bug. Pages 57-73 *in* R. F. Denno and H. Dingle, editors. Insect life history patterns: habitat and geographic variation. Springer-Verlag, New York New York, USA.

Environment Canada. 2009. Canadian climate normals 1971-2000. Retrieved January 15, 2010 from <http://climate.weatheroffice.gc.ca/climate_normals/index_e.html>.

Friberg, M., and C. Wiklund. 2010. Host-plant-induced larval decision-making in a habitat/host-plant generalist butterfly. Ecology 91:15-21.

Gotthard, K. 1998. Life history plasticity in the satyrine butterfly *Lasiommata petropolitana*: Investigating an adaptive reaction norm. Journal of Evolutionary Biology 11:21-39.

Grossmueller, D. W., and R. C. Lederhouse. 1985. Oviposition site selection: an aid to rapid growth and development in the tiger swallowtail butterfly, *Papilio glaucus*. Oecologia (Berlin) 66:68-73

Guo, K., S-G. Hao, O. J. Sun, and L. Kang. 2009. Differential responses to warming and increased precipitation among three contrasting grasshopper species. Global Change Biology 15:2539-2548.

Hutchinson, W. D., and D. B. Hogg. 1984. Demographic statistics for the pea aphid (Homoptera: Aphididae) in Wisconsin and a comparison with other populations. Environmental Entomology 13:1173-1181.

Ishihara, M. 1998. Geographical variation in insect developmental period: Effect of host plant phenology on the life cycle of the bruchid seed feeder *Kytorhinus sharpianus*. Entomologia Experimentalis et Applicata 87:311-319.

Johnson, J. P. 1941. Control of the hairy chinch bug. Bulletin - Connecticut Agricultural Experiment Station 445:372-373.

Lamb, R., G. Gerber, and G. Atkinson. 1984. Comparison of developmental rate curves applied to egg hatching data of E*ntomoscelis-americana* Brown (Coleoptera, Chrysomelidae). Environmental Entomology 13:868-872.

Leimar, O. 1996. Life history plasticity: Influence of photoperiod on growth and development in the common blue butterfly. Oikos 76:228-234.

Leonard, D. E. 1966. Biosystematics of the *leucopterus* complex of the genus *Blissus* (Hemiptera: Lygaeidae). Bulletin - Connecticut Agricultural Experiment Station 677:1-47.

Leslie, J. F. 1990. Geographical and genetic structure of life history variation in milkweed bugs (Hemiptera: Lygaeidae: *Oncopeltus*). Evolution 44:295-304.

Liu, H. J., and F. L. McEwen, 1979. The use of temperature accumulations and sequential sampling in predicting damaging populations of *Blissus leucopterus hirtus*. Environmental Entomology 8:512-515.

López, H., M. Nogales, and E. Morales. 2007. Habitat use and phenology of the large insular endemic grasshopper *Acrostira euphorbiae* (Orthoptera: Pamphagidae). Bulletin of Entomological Research 97:117-127.

Mailloux G., and H. T. Streu. 1981. Population biology of the hairy chinch bug (*Blissus leucopterus hirtus*, Montandon: Hemiptera: Lygaeidae). Annals of the Entomological Society of Quebec 26:51-90.

Masaki, S. 1978. Seasonal and latitudinal adaptations in the life cycles of crickets. Pages 72-100 *in* H. Dingle, editor. Evolution of insect migration and diapause. Springer-Verlag, New York, New York, USA.

Masaki, S., and W. Wipking. 1994. Insect and life cycle polymorphism: Introduction. Pages 1-3 *in* H. V. Danks, editor. Insect life history polymorphism: theory, evolution and ecological consequences of seasonality and diapause control. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Maxwell, K. E., and G. F. MacLeod. 1936. Experimental studies of the hairy chinch bug. Journal of Economic Entomology 29:339-343.

Morris, R. F. 1971. Observed and simulated changes in genetic quality in natural populations of *Hyphantria cunea*. Canadian Entomologist 103:893-906.

Nakai, T., and M. Takeda. 1995. Temperature and photoperiodic regulation of summer diapause and reproduction in *Pyrrhalta humeralis* (Coleoptera: Chrysomelidae). Applied Entomology and Zoology 30:295–301.

Niemczyk, H. D., R. A. J. Taylor, M. P. Tolley, and K. T. Power. 1992. Physiological Time-Driven Model for Predicting First Generation of the Hairy Chinch Bug (Hemiptera: Lygaeidae) on Turfgrass in Ohio. Journal of Economic Entomology 85:821-829.

Norris, R. F., E. P. Caswell-Chen, and M. Kogan. 2002. Chapter 4: Ecosystems and pest organisms. Pages 66-89 *in* Concepts in integrated pest management. Prentice Hall, Upper Saddle River, New Jersey, USA.

Nylin, S., and K. Gotthard. 1998. Plasticity in life history traits. Annual Review of Entomology 43:63-83.

Nylin S., and L. Svärd. 1991. Latitudinal patterns in the size of European butterflies. Holarctic Ecology 14:192-202.

Polivka, J. B. 1963. Control of hairy chinch bug, *Blissus leucopterus hirtus*, Mont., in Ohio. Ohio Agricultural Experiment Station Research Circular 122.

Potter, D. A. 1998. Destructive turfgrass insects: biology, diagnosis and control. Ann Arbor Press, Chelsea, Michigan, USA.

Pöyry, J., R. Leinonen, G. Soderman, M. Nieminen, R. Heikkinen, and T. Carter. 2011. Climate-induced increase of moth multivoltinism in boreal regions. Global Ecology and Biogeography 20:289-298.

Ragland, G. J., and J. G. Kingsolver. 2008. Evolution of thermotolerance in seasonal environments: the effects of annual temperature variation and life history timing in *Wyeomyia smithii*. Evolution 62:1345-1357.

Roff, D. 1980. Optimizing development time in a seasonal environment: the 'ups and downs' of clinal variation. Oecologia (Berlin) 45:202-208.

Sears, M. K., G. McEwen, G. Ritcey, and R. R. McGraw. 1980. Evaluation of insecticides for the control of hairy chinch bugs (Hemiptera: Lygaeidae) in Ontario lawns. Proceedings of the Entomological Society of Ontario 111:13-20.

Shama, L. N. S., and C. T. Robinson. 2009. Microgeographic life history variation in the alpine caddisfly: plasticity in response to seasonal time constraints. Freshwater Biology 54:150-164.

Showers, W. B. 1981. Geographic variation of diapause response in European corn borer. Pages 97-111 *in*: R. Denno, and H. Dingle, editors. Insect life history patterns: habitat and geographic variation. Springer-Verlag, New York, New York, USA.

Solbreck, C. 1978. Migration, diapause, and direct development as alternative life strategies in a seed bug, *Neacoryphus bicrucis*. Pages 195-217 *in* H. Dingle editor. Evolution of insect migration and diapause. Springer-Verlag, New York, New York, USA.

Solbreck, C. and B. Sillén-Tullberg.1981. Control of diapause in a "monovoltine" insect, *Lygaeus equestris* (Heteroptera). Oikos 36:68-74.

Sota, T. 1988. Univoltine and bivoltine life cycles in insects: a model with density dependent selection. Researches on Population Ecology 30:135-144.

Sota, T. 1994. Variation of carabid life cycles along climatic gradients: an adaptive perspective for life-history evolution under adverse conditions. Pages 91-112 *in* H. Danks, editor. Life cycle polymorphism: theory, evolution and ecological consequences for seasonality and diapause control. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology 46:336 -365.

Tashiro, H. 1987. Turfgrass insects of the United States and Canada. Cornell University Press, New York, New York, USA.

Tauber, M. J., and C. A Tauber. 1976. Larval diapause in *Chrysopa nigricornis* (Neuroptera: Chrysopidae): sensitive stages, critical photoperiod, and termination. Entomologia Experimentalis et Applicata 15:105–111.

Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. Seasonal Adaptations of Insects. Oxford University Press, Oxford, UK.

Traoré, L., J-G Pilon, F. Fournier, and G. Boivin. 2006. Adaptation of the development process of *Anaphes victus* (Hymenoptera: Mymaridae) to local climatic conditions across North America. Annals of the Entomological Society of America 99:1121-1126.

Trimble, R. M., and C. T. Lund. 1983. Intra- and interpopulation variation in the thermal characteristics of preadult development of two latitudinally diverse populations of Toxorhynchites rutilus septentrionalis (Diptera: Culicidae). Canadian Entomologist 115:659-662.

Trottier, R. 1971. Effect of temperature on the life-cycle of *Anax junius* (Odonata: Aeshnidae) in Canada. Canadian Entomologist 103:1671-1683.

Vittum, P., M. Villani, and H. Tashiro. 1999. Turfgrass insects of the United States and Canada, 2nd ed. Cornell University Press, Ithaca, New York, USA.

Wellwood, A., G. Nickerson, and J. Wetmore. 2002. Hairy chinch bug survey, demonstration and monitoring in New Brunswick, 2002. New Brunswick

Department of Agriculture, Fisheries and Aquaculture and New Brunswick Horticultural Trades Association, Fredericton, New Brunswick, Canada.

Wiklund, C., P. O. Wickman, and S. Nylin. 1992. A sexual difference in the propensity to enter direct/diapause development: a result of selection for protandry. Evolution 46:519-528.

Chapter 3: Local and Regional Level Variation in Sex Ratio, Wing Morph and Mensural Characters in *Blissus leucopterus hirtus* Montandon (Hemiptera: Blissidae)

3.1 Introduction

3.1.1 Morphometrics as a Measure of Diversity

In the late 18th century, Johan Christian Fabricius made an important contribution to ecology by relating morphometric structures to insect function in the environment (Tuxen 1967). Since then, the recognition of variability and specialization among forms has proven a powerful tool in ecological and evolutionary entomology (Gould and Johnston 1972, Hespenheide 1973, Debat et al. 2009). Morphometrics, the study of variation and change in form, was one of the first methods used to detect diversity among species and populations resulting from differences in ecological conditions across geographical ranges (Daly 1985). Although advances in genetic methodologies may now also be used to distinguish species and populations, in many cases, morphometric measurement remains an equally effective, practical way to discriminate between groups (Atchley and Hensleigh 1974, Sluss and Sluss 1982, Dujardin et al. 1999).

Morphometrics has been well researched in insects, which, due to their rigid exoskeletons, are easily collected and measured. (Daly 1985, Statzner et al. 2001). In addition to the ease of recording mensural characteristics, morphological polymorphisms are generally also simply identified and may offer insights into variation among

populations (Denno et al. 1985, Roff 1986). The morphometric diversity of individuals and populations is an indicator of environmental adaptation and can provide insight into local and/or regional environmental factors influencing size and shape. The relationship between habitat and life strategy has been well studied since even before 1977, when T.R.E. Southwood gave an address to the British Ecological Society in which he proposed the habitat templet model (Southwood 1977). This model became a foundational framework for the relation of life history strategy to selective forces within the environment, having now been cited over 1000 times. Southwood's ideas may have been inspired by an earlier presentation by Christer Solbreck, later published in 1978 (Solbreck 1978). Identifying variation among populations in morphometric characteristics at the local and/or regional level should increase awareness of the scale at which selective forces may be acting. Further evaluation of these patterns may also improve management practices through identification of correlative relationships between environmental parameters, population stability and individual fitness.

Blissus leucopterus hirtus Montandon (Hemiptera: Blissidae) (hairy chinch bug) is a widespread turfgrass and crop pest in Quebec and Atlantic Canada, and the northeastern United States (Vittum et al. 1999). It is capable of producing considerable damage to turf and lawns, an industry worth approximately 5 billion dollars a year in Canada (AAFC 2005). Effective management of this pest requires knowledge of its ecology, with emphasis on geographical specificity in life strategy acting as one pillar of a multi-faceted approach. Because both variation in size and polymorphism provide insight into environmental influences and adaptation by insects, an inventory of

morphometric characteristics among locally and regionally separated populations is necessary to justify and develop a more complete, regional and/or population based management strategy for *B. leucopterus hirtus*, particularly in determining populations establishment and other indicators of populations dynamics.

3.1.2 Size as a Measure of Diversity

Size is considered to be an important component of a complete life history model and is strongly related to development time and growth rate (Abrams et al. 1996, Nylin and Gotthard 1998, Danks 2006). The relationship between size, growth rate and environmental conditions is complex and involves a series of trade-offs (Danks 2006, Danks 2007). The importance of this relationship is underlined in seasonal environments, like those found across the range of *B. leucopterus hirtus*, where generational times must fit within variable time restrictions framed by periods of unsuitable conditions for development (Danks 1994, Abrams et al. 1996, Danks 2006, Danks 2007). Small size is typically considered a tradeoff for shorter development time and/or faster growth rate (Nylin and Gotthard 1998), as was observed in *B. leucopterus hirtus* in Chapter 2, and may be further influenced by factors including temperature (Sibley and Atkinson 1994) and food supply (Colbo and Porter 1979, Colbo and Porter 1981).

A very general rule of thumb is that adult size and fitness are positively correlated, with larger size indicating greater fecundity in females (Honěk 1993). However, there is also evidence that male size is important in selective mating (de Block and Stoks 2007, Couvillon et al. 2010). The optimal relative size appears to vary between

species and circumstance, responding to fluctuations in environmental conditions and/or population density (Kawecki 1993, Gilburn and Day 1994, Gage 1995). For example, although large, fast growing insects may acquire resources more easily and reach reproductive age more quickly, they may also be more susceptible to predation and/or symmetrical deviations than their smaller counterparts (Werner and Anholt 1993, Alcock 1995, Alcock 1996). Optimal size is therefore a balance among several traits including fecundity and survivability.

Numerous studies have demonstrated environmental and clinal variation in mensural characteristics for a range of organisms, including monkeys (Cardini et al. 2007), wolves (Milenković et al. 2010), mollusks (Schilthuizen et al. 2006), fish (Maderbacher et al. 2008) and among many insect groups: Blattodea (Slaney and Blair 2000), Coleoptera (Alibert et al. 2001), Orthoptera (Herrmann et al. 2010, Jannot et al. 2009), Diptera (Hernández-Ortiz et al. 2004), Hemiptera (Kaitala and Dingle 1992, Andersen 2000, Madjdzadeh and Mehrparvar 2009), Isoptera (Wilfert et al. 2006), Trichoptera (Shama and Robinson 2009) and Lepidoptera (Nylin 1994, Scriber 1994). These studies all relate variation in climate and geography as a driver for morphometric variation among populations and as an evolutionary strategy for optimizing structures and life traits to prevailing environmental conditions. Identifying similar variation in *B. leucopterus hirtus* should represent a fundamental first step in ascribing differences in size to varying selective forces across the insect's range, and could lead to improved interpretation of the suitability of local and regional habitat. For example, mean size of females within a population can provide information on the relative fecundity of those

individuals compared with other populations, and assist in the prediction of major infestations. This should hopefully assist pest managers to assess and predict population stability and robustness of *B. leucopterus hirtus*.

3.1.3 Sex Ratio

Both genetics and the environment may influence sex ratio (Uller et al. 2007). Although the proportion of males and females is approximately equal in most animal populations (Seger and Stubblefield 2002), selection will favour a biased sex ratio when the parental costs associated with producing each sex differ (Pen and Weissing 2002, Uller et al. 2007). Differences may also be seen when environmental conditions promote differential survival of the sexes (Wei 2008). Applying this knowledge, differences in sex ratio among populations may be indicative of variation among the selective forces operating upon those populations across a geographic range. The range of factors that may influences sex ratio from an environmental perspective temperature, photoperiod, and others, but the specific mechanism behind environmental sex determination are poorly understood in many species, including *B. leucopterus hirtus* (Schowalter 1996, Wei 2008). Sex ratio had been found to vary in Hemiptera (Groeters 1996, Ambrose 1999, Shahayarg and Sathiamoorthi 2002 and Cullen and Zalom 2005) and determination of any difference in sex ratio among populations of *B. leucopterus hirtus* at both the local and regional scale should provide a base from which to determine selective response of this pest to variable influences.

3.1.4 Wing Polymorphism

One easily visible measure of morphometric variation is polymorphic wing form, or the occurrence of multiple discrete wing forms within a single species. Polymorphic wing forms are common in several insect orders (Roff 1986). Southwood hypothesized that wing form was a response to habitat permanence (Southwood 1962) and predicted a correlation between habitat persistence and brachyptery, suggesting that macropterous forms should dominate in situations where recolonization was advantageous. This view has been reiterated many times since then (Southwood 1977, Solbreck 1978, Harrison 1980, Denno et al. 1985, Roff 1990, Denno 1994, Denno et al. 2001). However, dispersal can be advantageous even in stable environments, where it provides a stabilizing effect in periods of population fluctuation and prevents inbreeding (Hamilton and May 1977, Roff 1986).

The expression of wing morph is the result of a complex interplay between both environmental and genetic influences (Zera and Tiebel 1988, Fujisaki 1993, West-Eberhard 2003). As with size, the wing morph of an organism is the consequence of a series of trade-offs in reproductive potential, including dispersal and fecundity; a topic that has been extensively explored and reviewed (Denno et al. 1989, Roff 1994, Zera and Mole 1994, Zera and Denno 1997, Langellotto et al. 2000, Crnokrak and Roff 2002, Danks 2006, Danks 2007). Wing form development is at least partially regulated by hormones such as juvenile hormone (West-Eberhard 2003) and is generally related to flight capacity, but in some cases both macropterous and brachypterous individuals of the same species are flightless (as reviewed in Roff and Fairborn 1991). For example, recent

research on the firebug *Pyrrhocoris apterus* L. (Hemiptera: Pyrrhocoridae) suggests that flight capacity may also be influenced by muscle polymorphism (Socha and Šula 2006).

Some well-known predictors of wing form include insect density (Denno et al. 1985, Denno et al. 1991, Applebaum and Heifetz 1999), temperature (Pfenning et al. 2008, photoperiod (Harada et al. 2005), host plant nutrition (Denno et al. 1985) and combinations of these factors (Sasaki et al. 2002). However, other environmental and genetic factors may act alone or in combination to determine wing morphology (Denno et al. 1985, Langellotto and Denno 2001) and sexes within a single species may be influenced differently by various factors (Brisson et al. 2007). For example, in a study of wing dimorphism in *Prokelisia marginata* van Duzee (Homoptera: Delphacidae), macroptery in females was more highly correlated with density than in males (Denno et al. 1985). Denno et al. (1991) also reported differing rates of macroptery, among several species, between male and female planthoppers (Homoptera: Delphacidae) in relation to habitat persistence. Females responded to low densities in temporary habitats by showing increased levels of macroptery compared to those females in permanent habitats. However, males showed increased rates of macroptery in temporary habitats regardless of population density, suggesting that mate location in low density, temporary habitats may be an important determinate for wing form in some plant hoppers. Consequently, there is clear evidence of interplay between extrinsic factors such as density and intrinsic factors such as sex in the determination of wing form.

The ratio of brachypterous to macropterous individuals in both the male and female sex should provide insight into the permanence and stress on populations as a

result of environmental pressures. Comparing wing ratios at a local and regional level in *B. leucopterus hirtus* should provide insight into the scale at which wing ratio may be affected. This research should form the foundation required in order to attribute specific environmental influences to polymorphic characters, providing further insight into this pest's population dynamics and variability. Based on the relationship between population permanence and wing form, pest managers should be able to use wing form to estimate the stability of a particular population within a geographic locale.

3.1.5 Size, Sex and Polymorphism in *Blissus leucopterus hirtus*

The host range of *B. leucopterus hirtus* in Quebec and Atlantic Canada is climatically diverse (Chapter 1) and, based on implications of Southwood's habitat templet model (Southwood 1977), this may influence the morphometric characteristics of the insect in diverse ways. Because IPM emphasizes the need to account for variable life characteristics among populations of the same species, morphometrics may serve as a powerful tool to detect differences in life history among populations. Most of the limited references on *B. leucopterus hirtus* ascribe very general biological attributes to this important pest species and do not ascribe details below the species level. For example, texts typically list the adult as being 1mm in width and between 3.0-3.6 mm in length (Vittum et al. 1999, Potter 1998). These are general measurements, with no distinction made between males and females, long- and short-winged individuals or different populations. A more detailed study did report the head capsule widths, pronotum widths and length of terminal antennal segments in all instars of field collected samples of *B. leucopterus hirtus* (Mailloux and Streu 1981). However, the measurements were taken on

nymphs from a single population in Metuchen, New Jersey and these measurements did not include adults. The results were also not compared among populations from other locations (Mailloux and Streu 1981).

Although nearly all populations of *B. leucopterus hirtus* reported on in the literature have a combination of macropterous and brachypterous individuals, local dispersal of *B. leucopterus hirtus* has previously been assumed to be accomplished primarily through crawling rather than flight (Mailloux et Streu 1981). However, there is some evidence of movement due to either flight or air currents based on data from airplane sampling (Glick 1960, Mailloux et Streu 1981) and from personal observation by researchers (individual macropterous *B. leucopterus hirtus* found on clotheslines, clothing and other spaces difficult to reach in short periods through walking alone). Therefore, this research assumes that macropterous individuals are capable of some level of active or passive flight. Leonard (1966) reported brachyptery in *B. leucopterus hirtus* dominating the subspecies, with 64% of individuals displaying short winged pattern in one sampling collection (n=538). Wing form ratios in *Blissus leucopterus hirtus* have also been reported to vary between sexes with up to 50% more female macropters compared to male macropters (Mailloux and Streu 1981). Despite these individual records, information on wing form in *B. leucopterus hirtus* is still lacking.

In general, a high proportion of brachypterous individuals among insect populations is generally indicative of homogeneity and suitability of habitat, while long winged individuals are generally seen in patchy environments and in high numbers either preceding or following a recent migration (Southwood 1962, Southwood 1977, Solbreck

1978, Harrison 1980, Denno et al 1985, Roff 1990, Denno 1994, Denno at al. 2001, Danks 2006, Danks 2007). One would therefore expect to see the proportion of brachypterous *B. leucopterus hirtus* individuals increase over several years in an established population, until a point where resources become limited (e.g. a lawn is reduced in resource quality by intensive feeding) and dispersal becomes advantageous.

Previous observations of *B. leucopterus hirtus* by Mailloux and Streu (1981) showed that in an observed population with two cohorts per year, the proportion of macropters gradually decreased from April to June, rising rapidly during July as the spring cohort reached adulthood, and then declining again through August and September. This suggests dispersal or differential mortality by macropters during each of the two cohorts. Populations in this current study are defined by one cohort per year, but based on the previous study, one would expect the number of macropters to decrease through the entire season.

In an effort to understand the life strategy of *B. leucopterus hirtus* and gain insight into its population dynamics and ecology, Chapter 3 examines the variation in this insect`s morphology at a local and regional scale. Differences in morphological characteristics among populations should confirm the existence of adaptation and/or plasticity in this insect, and, building on previous work in this and other insect species, may offer insights into the environmental factors driving these differences. Sex ratio, wing form and morphology are compared at a local and regional scale, and these findings were further used to determine if a relationship exists between minimum development temperatures and egg development rate (characterized in Chapter 2) and adult size. This

should represent a fundamental basis for applying Integrated Pest Management practices based on population-specific knowledge of *B. leucopterus hirtus*, including age of population, fitness of population and likelihood of decolonization.

3.1.6 Research Hypotheses

This study characterized the morphometric characteristics of *B. leucopterus hirtus* to identify differences in these characteristics at the local and regional scale. Based on current knowledge of *B. leucopterus hirtus* and the habitat template theory, hypotheses are as follows:

1) Based on existing literature confirming the potential for sex ratio to fluctuate or vary among populations as a result of environmental influences, sex ratio in *Blissus leucopterus hirtus* differs among local populations in St. John's, NL with relation to collection site and density.

2) Based on the knowledge that wing form may be directly attributable to habitat favourableness, variability among sites in St. John's, NL produces differing wing form ratios in *Blissus leucopterus hirtus*. Wing form ratio is related to density within each collection season, and over the study period, the number of brachypters in local populations should increase as a reflection of the populations becoming increasingly established.

3) Based on variability among regional habitats (across Quebec and Atlantic Canada), *Blissus leucopterus hirtus* displays variation among: a) sex ratio, b) wing form and c) body size.

4) Based on a general relationship between growth rate and size in insects, *Blissus leucopterus hirtus* also displays a relationship between size ranking of adults and: a) mean threshold temperature for development, and b) mean egg development rate at various temperatures. Because the favourable growing season for *B. leucopterus hirtus* is

limited development rate should be rapid, and produce smaller individuals compared to those populations where this insect is exposed to longer periods favourable conditions.

3.2 Materials and Methods

3.2.1 Data Collection

3.2.1.1 Collection of Insects

3.2.1.1.1 Local Collection

In 2004 and 2005, ten sampling sites in St. John's, NL were used for this study. Four residential properties, four sites in St. John's city parks and two sites at AAFC's Cool Climate Crop Research Centre were selected (Fig. 3.1). Each site was placed into one of three categories (Low: <10 bugs per sample, Medium: 11-29 bugs per sample, High: ≥ 30 bugs per sample) based on the initial density of *B. leucopterus hirtus* as determined by a floatation sample as described below. Residential sites were identified during the course of a Canadian Broadcasting Corporation radio interview, by requesting St. John's residents to volunteer their infested properties for two years of study. The relative proximity of some sites reflects opportunistic sampling in areas where *B. leucopterus hirtus* were present, and the accessibility of these areas. Although all sites were located in St. John's, NL, there was some variability in land use and maintenance levels (Table 3.1). The visible concentration of *B. leucopterus hirtus* in areas of acute damage, combined with its tendency to aggregate with increasing age, suggests that

populations do not mix readily throughout the growing season. This assumption was be followed in the comparison of locally separated populations.

Table 3.1. Primary land use, turf maintenance level and initial density of *B. leucopterus hirtus* at 10 study sites within St. John's, NL

Site	Density of *B. leucopterus hirtus**
A	medium
B	high
C1†	medium
C2†	medium
D	Low
E	low
F	Low
G	Low
H	low
I	medium
J	high

*Density classes: low: <10 bugs per sample, medium: 11-29 bugs per sample, high: ≥ 30 bugs per sample

† Due to the destruction of site C1 in late 2004, C1 was replaced by C2, a site with similar characteristics, in 2005

Sampling of *B. leucopterus hirtus* was conducted at approximately weekly intervals for 18 weeks per year from early June until late October in 2004 and 2005. At the onset of the project in 2004, delays in locating sites of low density *B. leucopterus hirtus* prevented a full 18 week sampling regime at some sites. In 2005, all sites were sampled 18 times. Based on personal observations, *B. leucopterus hirtus* appeared to be most active and visible under sunny conditions and warm temperatures. Therefore, whenever possible, sampling was conducted on warm, sunny days.

In the spring of 2004, a plot of approximately 2 m^2 was selected for sampling at each site. The sampling process started each week with a visual search of the plot to determine the location of highest *B. leucopterus hirtus* density. Floatation sampling was used to collect the insects. This method was chosen following an experimental comparison of sampling methods as part of the current study (Appendix C) and because this method was used in similar studies elsewhere (Wellwood et al. 2002). At each sampling site, a PVC pipe (d=15 cm and h=20 cm) with a beveled edge was inserted approximately 2.5 cm into the ground using a mallet. The cylinder was then filled ¾ full with water.

FIGURE 3.1. Location of ten local study sites in St. John's, NL. Sites are designated with an identifying letter. Due to the destruction of site C1 in late 2004, C1 was replaced by C2, a site with similar characteristics, in 2005. Refer to Table 3.1 for additional description of sites.

Blissus leucopterus hirtus that floated to the surface within a 10 minute period were collected using a fine mesh tea strainer, with water replenished as required. The turf within the cylinder was agitated using a knife at 2 min, 5 min and 8 min. The insects were then placed in 500 mL plastic containers labeled with date, time and location, taken to the laboratory and frozen for later assessment. After thawing, *B. leucopterus hirtus* were removed from the debris with the aid of a magnifier light, tweezers and a fine artist's-paintbrush. Specimens were placed on filter paper in Petri dishes and identified to life stage using a stereomicroscope. Adult sex and wing form were also recorded. All specimens were then preserved in sealed glass vials containing 70 % ethanol and labeled with pertinent sample information.

3.2.1.1.2 Regional Collection

In May 2008, *B. leucopterus hirtus* spring adults were collected from twelve sites in Atlantic Canada. Local populations, with the exception of those in New Brunswick, were sampled from sites within a few kilometers of each other. In New Brunswick, based upon a local recommendation, two populations were sampled in Fredericton, and one 82 km away, close to Saint John, NB. 'Regional' areas were defined by provincial boundaries, with province names serving as identifiers for groups of local sites. In total, the sampling sites included three in Quebec City (PQ), two in Fredericton (NB), one in Saint John (NB), three in the Annapolis Valley (NS) and three in St. John's (NL) (Fig. 3.2). The three populations selected in St. John's in 2008 were different than the 10 sites sampled from 2004-2005. Of these additional sites, all three were residential lawns. Two of the residential lawns were well maintained (although damaged by *B. leucopterus*

hirtus) and the other sampling site had low level of care. The reason for the alternate choice in sampling site in St. John's (different from the 2004-2005 study) was to avoid possible influences on the populations from previous years of sampling, including the potential for population depletion.

Individuals were collected using a combination of hand sampling and vacuum sampling in areas identified by local entomologists as inhabited by *B. leucopterus hirtus* populations. Sampling methods were chosen for based on speed, efficacy and minimal damage to turf (Appendix C). Over the 14 day collection trip, captured individuals were placed in plastic containers with grass and plant debris from the local collection area and kept in a cooler above ice packs that were refrozen daily. Upon arrival in St. John's, the adult bugs were transferred into site-specific large sized plastic *Ziploc*® Snap 'n Seal containers, with each container considered an individual colony.

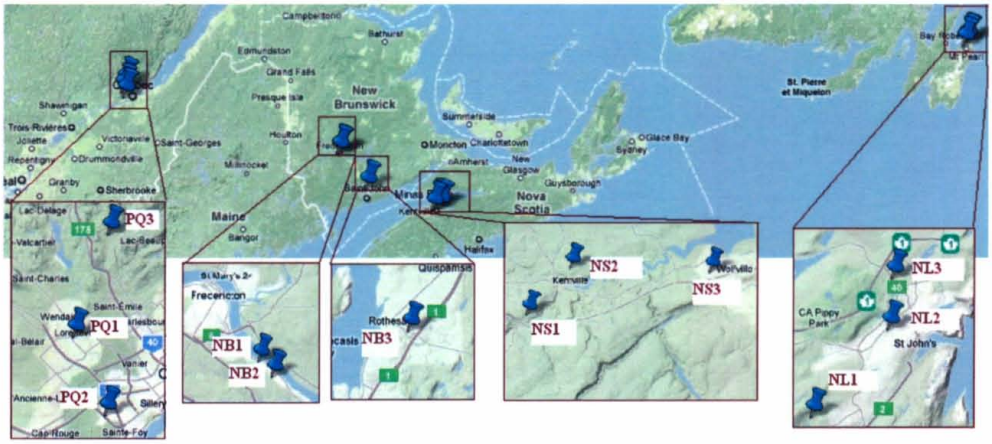

FIGURE 3.2. Location of twelve study sites across Atlantic Canada. Location of twelve study sites in Quebec and Atlantic Canada, at each of which *Blissus leucopterus hirtus* were collected over a two week period in May 2008, using a combination of hand sampling and vacuum sampling. Sampling locations were identified by local entomologists as inhabited by *B. leucopterus hirtus* populations. Sites are labeled in enlarged map sections.

The containers were modified by replacing the middle section of the lid with a 0.2 mm mesh that promoted airflow but prevented the bugs from escaping. The size of mesh chosen was based on the minimum 1st instar head capsule width of 0.21 mm recorded by Mailloux and Streu (1981). Any plant material and debris in the containers was removed and all colonies were fed stem sections from green house reared *Zea mays* var. polka (Veseys Seed Company, Charlottetown, PEI) between the 3rd and 4th leaf stage. The colonies were placed into a Conviron® growth chamber maintained at 20°C±1°C, with a 16:8 hour photoperiod at 100% relative humidity. Several individuals were collected from each colony and sent to AAFC Ottawa for identification. All samples submitted were positively identified as *B. leucopterus hirtus*. Colonies were monitored daily, and many of the adults were isolated as part of a study on egg development. Following completion of oviposition or natural death, all adults were placed in a glass vial filled with 70% ethanol and labeled with population of origin and collection date.

3.2.1.2. Collection of Traditional Morphometric Data

Adult *B. leucopterus hirtus* were removed from the glass vials and individually examined under a stereomicroscope. Their wing form and sex were recorded, and measurements were taken manually using an ocular micrometer under 40 x magnification. Locations of measurement points are provided (Fig. 3.3) along with descriptions of measurements taken (Table 3.2). The presence of aberrations was noted when visible deformities were obvious. Examples of aberrations observed included reduction in the number of antennal segments (not including recent breakage), fusing of

the antennal segments, absence of tarsi (not including recent breakage) and obviously deformed wing shapes (corkscrew shape, obvious disproportion to opposite wing).

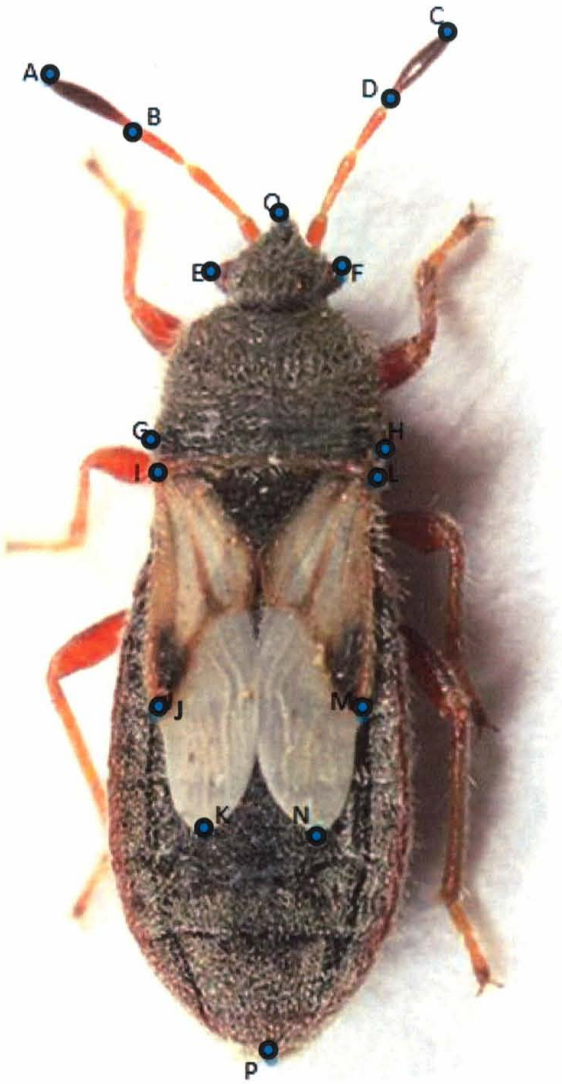

FIGURE 3.3. Measurement points (indicated by circles) for morphometric measurements of *Blissus leucopterus hirtus*. Copyright © 2008 Tom Murray. Used with permission. See Table 3.2 for an explanation of lettering.

TABLE 3.2. Morphometric measurements collected in study of *Blissus leucopterus hirtus.*

Measurement	Name	Description
A→B	left antenna	Length of left antennal segment iv from tip to articulation with segment iii.
C→D	right antenna	Length of right antennal segment iv from tip to articulation with segment iii.
E→F	eye to eye	Width between outer margins of eyes
G→H	thorax	Greatest width between outer margins of thorax
I→J	left corium	Greatest length of left wing corium from insertion on thorax to wing membrane
L→M	right corium	Greatest length of right wing corium from insertion on thorax to wing membrane
I→K	left wing	Left wing length from thorax articulation to tip of membrane
L→N	right wing	Right wing length from thorax articulation to tip of membrane
O→P	total length	Length of insect's body along an axis from tip of vertex to tip of abdomen.

3.2.2 Data Analysis

All statistical analysis was conducted using JMP® 7.0.1 and JMP® 8.0.2.2 (SAS Institute Inc., Cary, NC, USA)

3.2.2.1. Analysis of locally collected data

Data for sex and wing form of adult *B. leucopterus hirtus* collected from 10 local sites in St. John's were divided into four collection periods based on high numbers of adults during these times: Spring 2004, Fall 2004, Spring 2005 and Fall 2005. Because of overwintering of this insect, Fall 2004 and Spring 2005 should represent the same cohort, but they were examined separately to determine if differential survival of overwintering adults occurred. To compensate for a potential skew in density calculations for each collection period, the total number of adult insects collected was divided by the total number of sampling days at each site, so data are in units of 'number of bugs per sampling day'.

The proportion of males and females at each site was compared for each of the four collection periods using Pearson's Chi-Square test, with collection period serving as the factor determining the number of females. Site data within each collection season was then pooled and Pearson's Chi-Square was used to determine whether the ratio of males and females depended on the factor of collection seasons. Pearson's Chi-Square was then use to determine of the F:M ratio in each collection season varied from F:M 1:1. Wing ratio was then compared by the factor of local site in each collection season using a Pearson's Chi-Square test. The proportion of brachypters in each cohort was compared to

the mean density of adult *B. leucopterus hirtus* per sample using a bivariate fit plot and correlation analysis to determine if wing form was related to density.

3.2.2.2 Analysis of regionally collected data

Sex ratios were calculated for locally and regional separated populations across Quebec and Eastern Canada as well as the entire study region in order to allow inter-site, inter-region and inter-study comparisons using Pearson's Chi-Square statistics. In these cases sites and regions served as the primary factors in determining sex ratios. The same approach as above was applied to examine the proportion of brachypterous and macropterous individuals. Because of potential error inherent in manually measuring and recording morphological characteristics (Daly 1985), an assessment of measurement error was conducted using 42 individual specimens according to the method described by Arnqvist and Mårtensson (1998). Using their protocol, nine morphometric characteristics were measured on 42 individual specimens and each individual was placed in a separate vial. The vials were shuffled and the insects were re-measured in a random order. A one-way ANOVA was used to compare variation between repeated measures (error within) to variation among individuals (error among).

A multivariate scatterplot matrix was created to identify sets of measurements with high multicollinearity, including symmetric characteristics, so that they could be eliminated in further analysis. A one-way ANOVA was used to determine if significant differences in character length existed among sites for either long winged or short winged individuals. For each character, mean distances between measurement points at each site

were then ranked from greatest distance to shortest distance. The rankings for multiple characters for each site were averaged to produce an overall size rank. The rankings were then compared with categorized density of individuals at each site to determine if there was a relationship between density and size. In order to test for significant differences in morphological characteristics between males and females and between brachypters and macropters, site data were pooled and compared by one-way ANOVA.

3.3 Results

3.3.1 Variation in Sex Ratio and Wing Form Ratio

The variation among measurements in this study was primarily attributed to differences among individuals and not measurement error (Appendix E). There was a high rate of physical deformity in the majority of populations sampled (Appendix F). A additional multivariate discriminant analysis was performed on the data to determine if the method was successful at differentiating among populations at either the local or regional level but success was limited (Appendix G).

3.3.1.1 Local variation in sex ratio and wing form ratio

A total of 1460 adult individuals were collected in St. John's, NL over four collection periods in 2004-2005: 169 (Spring 2004), 534 (Fall 2004), 148 (Spring 2005) and 609 (Fall 2005). Sex ratio did not vary significantly among local sites in St. John's in any collection period: Spring 2004: X^2=9.489, df=9, p=0.3934, Fall 2004: X^2=11.032, df=7, p=0.1372, Spring 2005: X^2=7.990, df=7, p=0.3335 and Fall 2005: X^2=8.914, df=8, p=0.3496. Site data were, therefore, pooled and sex ratios compared among sampling

seasons. There was no significant change in proportion of males and females over the four sampling seasons (X^2=0.218, df=3, p=0.9747).

However, when compared against a predicted ratio of 1:1, there was a significant deviance from this ratio is both Fall 2004 and 2005 (Spring 2004: X^2=0.7160, df=91, p=0.3975, Fall 2004: X^2=5.4607, df=1, p=0.0194, Spring 2005: X^2=0.9730, df=1, p=0.3239 and Fall 2005: X^2=6.1100, df=1, p=0.0134). In both Fall 2004 and Fall 2005, more males were collected than females, indicating evidence of either differential mortality or dispersal by females. Combining data from all sites over all sampling seasons resulted in a ratio of females to males of 1:1.19, which is significantly different from 1:1 (X^2=9.8620, df=1, p=0.0017).

The proportion of brachypterous individuals varied significantly among sites for three collection seasons (Spring 2004: X^2=17.082, df=9, p=0.0475, Fall 2004: X^2=60.364, df=7, p <0.0001, Spring 2005: X^2=18.661, df=7, p=0.0093. There was no difference among sites in Fall 2005 X^2=9.820, df=8, p=0.2779, a period during which brachypterous individuals were abundant at all sites.

The ratio of brachypters to macropters differed significantly between collection periods for both males (X^2=71.987, df=3, p <0.0001) and females (X^2=85.119, df=3, p <0.0001; Table 3.3). However, there was no significant difference in wing form between Fall 2004 and Spring 2005 (two sampling periods assumed to represent the same cohort) in males (X^2=0.027, df=1, p=0.8692) or females (X^2=0.183, df=1, p=0.6691), and therefore no evidence of differential overwinter mortality between brachypterous and

macropterous individuals. There were also significantly more male brachypters in Fall 2004 (X^2=14.2604, df=1, p=1.0638), Spring 2005 (X^2=4.2000, df=1, p=0.404) and Fall 2005 (X2=6.3380, df=1, p=0.118).

The proportion of brachypters at each site was compared to the mean density of adult *B. leucopterus hirtus* per sample in each cohort to determine if wing form was related to density. In this analysis, the data for overwintered spring 2005 adults were combined with Fall 2004, as these bugs were from the same cohort and showed no difference in wing ratio. There was no clear trend and no significant relationship between density of bugs per sample and wing form for any of the three cohorts (Fig 3.4).

TABLE 3.3. Number of macropterous (M) and brachypterous (B) *Blissus leucopterus hirtus* (females and males) collected in four sampling periods in St. John's, NL. The distribution of brachypters and macropters is significantly different among collection seasons (X^2=85.119, df=3, p<0.0001).

Collection Season	**Males**			**Females**		
	M	**B**	**Ratio (M:B)**	**M**	**B**	**Ratio (M:B)**
Spring 2004	38	52	1:1.37	37	42	1:1.14
Fall 2004	65	229	1:3.52	85	155	1:1.82
Spring 2005	17	63	1:3.70	26	42	1:1.62
Fall 2005	21	314	1:14.95	20	254	1:12.7

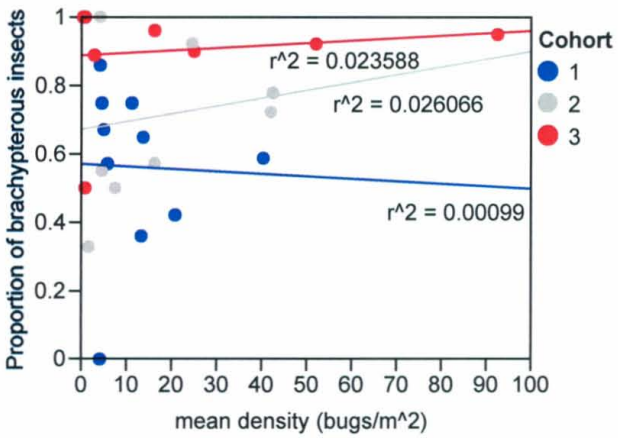

FIGURE 3.4. The proportion of brachypterous individuals in relation to the approximate density of *B. leucopterus hirtus* at each collection site for three cohorts, calculated by dividing the total number of bugs per site/number of sampling days. Cohort 1 is Spring 2004, Cohort 2 is Fall 2004 and Spring 2005, and Cohort 3 is Fall 2005.

3.3.1.2 Regional variation in sex ratio and wing form

In Spring 2008, 1351 *B. leucopterus hirtus* adults were collected from twelve sites across Atlantic Canada. There was no significant difference among the sex ratios at any of the sites (X^2=14.625, df=11, p=0.2003, Table 3.4), nor among sites within the same province (PQ: X^2=1.043, df=2, p =0.5938, NB: X^2=0.471, df=2. 0.471, p=0.2003 NS: X^2=2.028, df=2, p=0.3627, NL: X^2=0.426, df=2, p=0.8083). The site specific sex ratios did not vary from 1:1, except in the case of NB2 (X^2=6.6931, df=1, p=0.0097) and NS2 (X^2=10.9206, df=1, p=0.0010). When data was pooled by province, there was a significant difference in sex ratios among provinces (X^2=10.695, df=3, p=0.0135). There was no deviation from 1:1 sex ratio in NL (X^2=1.0901, df=1, p=0.2965) and PQ (X^2=2.1985, df=1, p=0.1381) but there were statistically more males than females in NB (X^2=7.5294, df=1, p=0.0061) and NS (X^2=9.6604, df=1, p=0.0019). The pooled sex ratio

for all samples collected was F:M=1:1.19, which varied significantly from an expected ratio of 1:1 across the entire region (X^2=9.8620, df=1, p=0.0017).

TABLE 3.4. Number of females (F) and males (M) *Blissus leucopterus hirtus* collected from 12 sites across Quebec and Atlantic Canada. The distribution of females and males is not significantly different among sites (X^2=14.625, df=11, p=0.2003). When sites were pooled by province, there was a significant difference among F:M ratio among regions (X^2=10.695, df=3, p=0.0135).

	By Site			**By Province**		
Site	Female	Male	Ratio (F:M)	Female	Male	Ratio (F:M)
PQ1	49	48	1:0.98	143	119	1:0.83
PQ2	34	25	1:0.74			
PQ3	60	46	1:0.77			
NB1	34	37	1:1.09	240	304	1.26
NB2	176	228	1:1.30			
NB3	30	39	1:1.30			
NS1	43	46	1:1.07	180	244	1.35
NS2	111	166	1:1.50			
NS3	26	32	1:1.23			
NL1	11	14	1:1.27	50	61	1.22
NL2	35	40	1:1.14			
NL3	4	7	1:1.75			
Combined	613	728	1:1.19			

The proportion of brachypterous and macropterous individuals varied significantly among sites (X^2=30.698, df=11, p=0.0012, Table 3.5). The ratio of brachypterous to macropterous individuals overall was 4.33:1 in females and 5.39:1 in males. However, a Pearson Chi-Square revealed no significant effect of sex on wing morph (X^2=2.260, df=1, p=0.1328).

TABLE 3.5. Number of macropterous (M) and brachypterous (B) *Blissus leucopterus hirtus* collected from 12 sites across Quebec and Atlantic Canada. The distribution of brachypters and macropters is significantly different among sites (X^2=30.698, df=11, p=0.0012).

Site	Macropters	Brachypters	Ratio (M:B)
PQ1	10	87	1:8.70
PQ2	17	42	1:2.47
PQ3	7	80	1:2.96
NB1	7	72	1:9.29
NB2	75	333	1:4.44
NB3	14	55	1:3.93
NS1	15	76	1:5:07
NS2	51	229	1:4.49
NS3	0	58	n/a
NL1	2	23	1:11.5
NL2	14	61	1:4.36
NL3	2	9	1:4.50

Mensural characteristics were compared among sites using a one-way ANOVA. There was a significant difference among the twelve sites for both brachypterous and macropterous characters (Fig 3.5, Fig 3.6). Nova Scotia and Quebec generally had larger bugs, while New Brunswick and Newfoundland individuals were smaller by comparison.

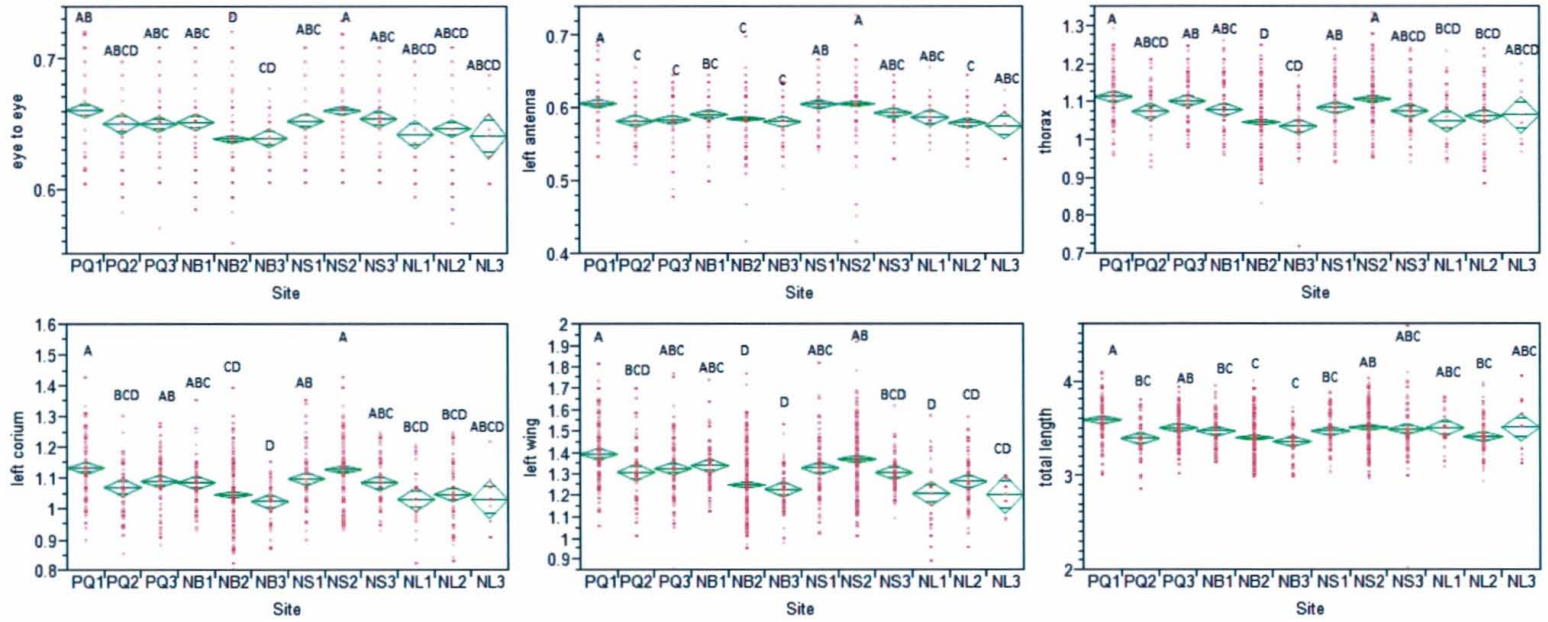

FIGURE 3.5. One-way ANOVA comparison of eye to eye width ($F(11, 1098)=11.2144$, $p<0.0001$), terminal left antenna length ($F(11, 1098)=13.7673$, $p<0.0001$), thorax width ($F(11, 1098)=4.0597$, $p<0.0001$), left corium length ($F(11, 1098)=15.8661$, $p<0.0001$), left wing length ($F(11, 1098)=14.3804$, $p<0.0001$), and total length ($F(11, 1098)=8.3242$, $p<0.0001$) in brachypterous *B. leucopterus hirtus* collected from twelve sites across Quebec and Atlantic Canada in Spring 2008. The mean line across the middle of each diamond represents the group mean. The top and bottom of each diamond represent the 0.95 confidence interval for each group. Diamond overlap marks appear as lines above and below the group mean. Overlapping marks indicate that the two group means are not significantly different at the given confidence level. Group data sharing the same letter above the data are not significantly different based on a Tukey HSD test.

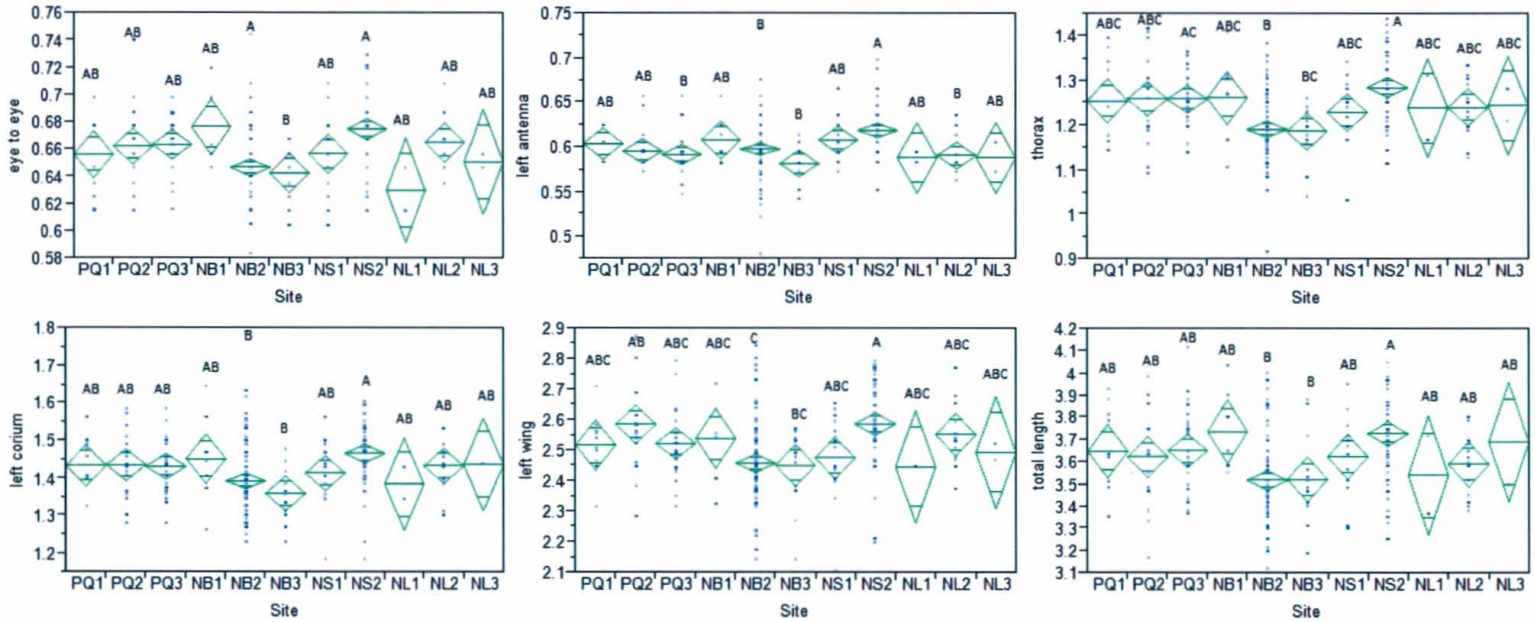

FIGURE 3.6. One-way ANOVA comparison of eye to eye width ($F(10, 220)=4.3688$, $p<0.0001$), terminal left antenna length ($F(10, 220)=3.4851$, $p=0.0003$), thorax width ($F(10, 220)=5.3871$, $p<0.0001$) left corium length ($F(10, 220)=3.0239$, $p<0.0001$), left wing length ($F(10, 220)=3.0231$, $p=0.0013$) and total length ($F(10, 220)=4.3871$, $p<0.0001$) in macropterous *B. leucopterus hirtus* collected from twelve sites across Quebec and Atlantic Canada in Spring 2008. The mean line across the middle of each diamond represents the group mean. The top and bottom of each diamond represent the 0.95 confidence interval for each group. Diamond overlap marks appear as lines above and below the group mean. Overlapping marks indicate that the two group means are not significantly different at the given confidence level. Group data sharing the same letter above the data are not significantly different based on a Tukey HSD test.

3.3.2 Relationship Between Size, Threshold Temperature for Egg Development and Egg Development Rate

To facilitate comparison of egg development rates and threshold temperature for development with insect size in each populations, sites were ranked from maximum mean character length/width (rank=1) to minimum mean character length/width (rank=11* (Table 3.6)) for both brachypterous and macropterous forms. The length/width rankings of each character for a particular site were then averaged to give an overall size ranking for insects at each site (Table 3.6).

TABLE 3.6. Size rankings for brachypterous and macropterous *B. leucopterus hirtus* for twelve sites in Atlantic Canada.

Brachypterous			Macropterous		
Overall rank	*Site*	*Mean size ranking (1=largest)*	*Overall Rank (1=largest)*	*Site*	*Mean size ranking*
1	PQ1	1.0	1	NS2	1.3
2	NS2	2.0	2	NB1	2.0
3	NS1	4.3	3	PQ2	4.2
4	PQ3	5.0	4	PQ1	5.0
5	NB1	5.1	5	PQ3	5.7
6	NS3	5.3	6	NL2	5.8
7	PQ2	7.7	7	NL3	6.2
8	NL2	8.8	8	NS1	7.3
9	NL3	9.3	9	NB2	8.7
10*	NB2	9.7	10	NL1	9.8
10*	NL1	9.7	11	NB3	10.3
11	NB3	11.2	†		

* NB2 and NL1 had the same size ranking and therefore, the same overall rank.
† No intact macropterous individuals were found at NS3.

There was no correlation between average size ranking of brachypterous individuals and either threshold temperature for egg development (r^2 (9)=0.1284, p=0.2527) or egg growth rate for eggs reared at 20°C (r^2(10)=0.0048, p=0.8308), 25°C (r^2(10)=0.1302, p=0.2493), or 30°C (r^2(10)=0.2366, p=0.1088)(Fig. 3.7). Similarly, there was no correlation between average size ranking of macropterous individuals and either threshold temperature for egg development (r^2(9)=0.0472, p=0.5213) or egg development rate at 20°C (r^2(9)=0.0016, p=0.9067), 25°C (r^2(9)=0.1355, p=0.2654) or 30°C (r^2(10)=0.0058, p=0.8242).

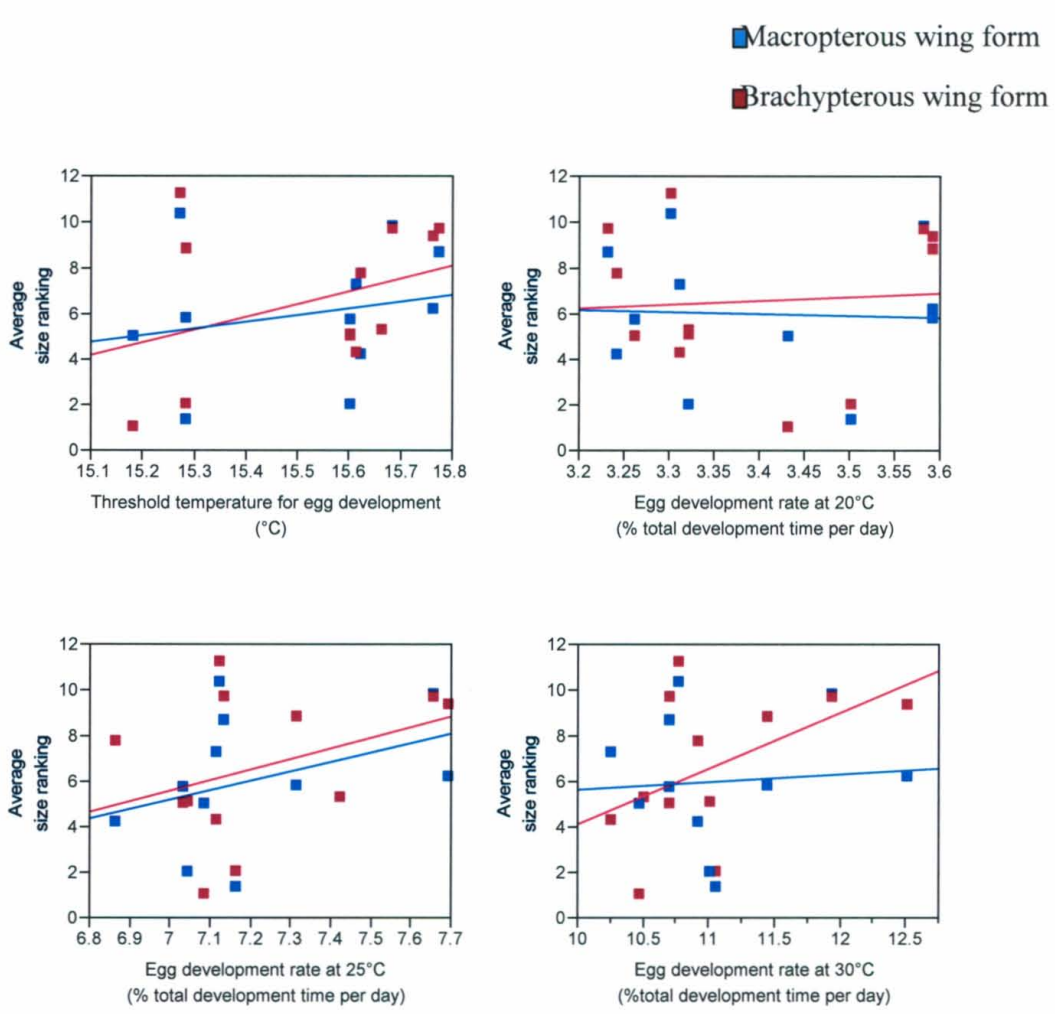

FIGURE 3.7. The relationship in *B. leucopterus hirtus* between average size ranking in brachypterous threshold temperature for egg development ($r^2(10)=0.1284$, $p=0.2527$) and brachypterous egg development rate at 20°C ($r^2(10)=0.0048$, $p=0.8308$), 25°C ($r^2(10)=0.1302$, $p=0.2493$), and 30°C ($r^2(10)=0.2366$, $p=0.1088$) and relationship the relationship between average size ranking in macropterous and threshold temperature for egg development ($r^2(9)=0.0472$, $p=0.5213$) and macropterous egg development rate at 20°C ($r^2(9)=0.0016$, $p=0.9067$), 25°C ($r^2(9)=0.1355$, $p=0.2654$), and 30°C ($r^2(10)=0.0058$, $p=0.8242$) individuals from sites in Quebec and Atlantic Canada. Threshold temperature for development is based on simulations performed in Chapter 2 using iteration within a non-linear model of the hyperbolic equation regression, and each point of the graph is derived from the combined data at each site. Similarly, egg development rate is based on the average rate at each site.

3.4 Discussion

3.4.1 Sex Ratios

The proportion of males and females was similar at all sites in St. John's (2004-2005) in each of the four collection periods, and at all sites across Quebec and Atlantic Canada in 2008. The ratio across the entire study range of F:M 1:1.19. The ratio obtained in this study is comparable to the ratio of: 1:1.12 obtained by Mailloux and Streu in their study on *B. leucopterus hirtus* (Mailloux and Streu 1981). Results from this study are comparable to other Hemipteran M:F sex ratios, including: *Nysius huttoni* White (Hemiptera: Lygaeidae) 1:1.05 (Wei 2008), *Oncopeltus fasciatus* Dallas (Hemiptera Lygaeidae) 1:1 (Sauer and Feir 1973), *Nysius ericae* Schilling (Hemiptera: Lygaeidae) 1:1.13 -1:1.18 (reviewed by Wei 2008), *Cyclopelta obscura* Lepeletier and Serville (Hemiptera: Pentatomidae) 1:1 (Zhang 1985) and *Eurydema gebleri* Kolenati (Hemiptera: Pentatomidae) 1:1 (Zhang 1985). In their attempt to explain the relatively high number of males compared to females, Mailloux and Streu suggested that a higher number of males may act to counter virgin female isolation, the effects of which include lower egg productivity and increased longevity. More males effectively provides more chances for copulation and increasing oocyte production (Sweet 1963, Mailloux and Streu 1981).

Within the St. John's, NL populations, F:M ratios were significantly male biased in Fall 2004 and Fall 2005. This indicates either differential mortality or dispersal by females during the summer season. Previous studies of several predatory Hemipteran species (*Anthocoris tomentosus* Péricart (Hemiptera: Anthrocoridae), *A. antevolens* White

(Hemiptera: Anthrocoridae) and *A. brevis* Uhler (Hemiptera: Anthrocoridae) showed a strong overwintering mortality in males (Horton et al. 1998, Horton and Lewis 2000). However, there was no evidence of differential overwintering mortality of males of *B. leucopterus hirtus* in the populations studied. Apart from the potential for differential mortality or dispersal over time, the stability of the sex ratio across the entire range indicates that either genetic influences on sex ratio are highly preserved and/or environmental differences were insufficient to result in differential fitness of either sex in these patchy environments. The implication of these results is that environment has not differentially selected for sex ratio at either the local or regional scale in *B. leucopterus hirtus* and, therefore, does not appear to be an effective indicator of population variation within this species.

3.4.2 Wing Form Ratios

Previous observations of *B. leucopterus hirtus* by Mailloux and Streu (1981) showed that the proportion of macropters gradually decreases from April to June, rises rapidly during July as the spring cohort reached adulthood, and declines again through August and September. This suggests dispersal or differential mortality by macropters during each of the two cohorts. Unlike Mailloux and Streu's research (1981), the current study found wing form ratio over multiple weeks within a single collection year to be fairly consistent and, therefore, does not support earlier findings. However, based on the combined weekly samples taken from three cohorts (Spring 2004, Fall 2004 - Spring 2005 and Fall 2005) there was a progressive increase in brachyptery in both males and females over three generations studied.

An increase in short winged individuals is generally indicative of homogeneity and suitability of habitat, since long winged individuals are generally seen in patchy environments and in high numbers either preceding or following a recent migration (Danks 2006, Danks 2007). Southwood's habitat templet model postulated that wing form was a response to habitat permanence (Southwood 1962) and predicted a correlation between habitat persistence and brachyptery, suggesting that macropterous forms should dominate in situations where recolonization was advantageous (Southwood 1977, Solbreck 1978, Harrison 1980, Denno et al. 1985, Roff 1990, Denno 1994, Denno et al. 2001). Based on the shifting wing form ratios over three cohorts in this study, it is possible that the sites sampled were sufficiently suitable that, over time, *B. leucopterus hirtus* devoted fewer resources to recolonization. Although the age of the populations at the start of this study in 2004 is unknown, many sampling locations were selected based on reports of recent infestation. It may be that the degree of brachyptery in the populations increased over time as the populations became increasingly established in the study areas. However, with limited evidence of a direct relationship between wing form variation and probability of dispersal, and ontological period when wing form is determined, conclusions are limited at this time.

Wing form is likely influenced by a combination of environmental conditions (Zera and Tiebel 1988, Fujisaki 1993, West-Eberhard 2003). These influences are especially difficult to tease out on account of their dynamic and intertwined nature (Denno 1994, Gatehouse 1994). Previous studies and reviews have identified multiple factors influencing wing ratios, including density (Denno et al. 1985, Denno et al. 1991,

Applebaum and Heifetz 1999), temperature (Pfenning et al. 2008), photoperiod (Harada et al. 2005), host plant nutrition (Denno et al. 1985) and combinations of these factors (Sasaki et al. 2002). Previous work on *Dimorphopterus japonais* Hidaka (Heteroptera: Lygaeidae) suggested that crowding could produce increased rates of macroptery, but that physical contact between nymphal instars appeared to be necessary to produce increased incidence of long-winged bugs (Sasaki et al. 2002). Denno et al. (1991) also concluded that density was the most important factor in determining wing form in most species of planthoppers. In addition to weather and climate, there are several other ecological influences inherent to the environment of *B. leucopterus hirtus*. Although seemingly uniform, lawns and other green spaces can vary in plant composition and health, abundance of natural enemies (e.g. *Geocoris punctipes* Say (Hemiptera: Geocoridae) and endophytic fungi (e.g. *Acremonium* spp)), protective spaces and canopy presence as well as level of maintenance. Although this study did not address all of these factors specifically, it is likely that a combination of population and environmental factors were responsible for any variation observed (although non-significant in some regions). If flight is possible, the variable wing ratio among sites observed at both the local and regional level, and over three cohorts, suggests that wing form may be a valuable indicator of discrete environmental influences among populations. Extensive modeling using a wide range of in-situ and ex-situ populations would be necessary to elucidate the specific determinants of wing morph. For example, a true measurement of density was difficult within the context of a field study such as this. It is possible that the methods used in this paper were simply not robust enough to detect influences such as density on wing form.

3.4.3 The Interrelationship of Sex Ratio and Wing Form

A relationship between sex and wing form ratios is not uncommon among insects, and may be linked to several factors including habitat permanence and quality (Julliard 2000). The existence of a dimorphic wing form in both sexes suggests a fitness tradeoff between wing production and some other life function (Denno et al. 1991). The majority of research concerning reproductive tradeoffs has been conducted with female insects in which size, strength and/or longevity are traded off for egg quality and/or production (Tanaka 1976, Roff 1994, Denno 1994, Zera 1984). However, research has shown a fitness tradeoff in mating success in male Hemipterans, including *Prokelisia dolus* Wilson (Hemiptera: Delphacidae) in which a greater sperm load was recorded in brachypterous individuals (Langellotto et al. 2000) and the false chinch bug *Cavelerius saccharivorus* Okajima (Heteroptera: Lygaeidae) due to faster maturation of brachypterous insects (Fujisaki 1993). The results from this study showed that males were more commonly collected in Fall 2004 and Fall 2005 than females and that females were more likely to be macropterous. The relatively higher occurrence of macroptery in females over males in 2004-2005 suggests that dispersal by flight, if it occurs, is relatively more advantageous to females than males and that due to a lower number of females in fall, it is likely they are either dispersing or experiencing earlier mortality. However, since a higher incidence of female macropters versus male macropters was not found in other regions across the range in Spring 2008, it is difficult to make inferences regarding the interplay between sex, wing form and fitness across the entire region.

The quality of habitat at each of the population sites in this study was not evaluated, but future correlative studies would provide a more robust exploration of the mechanisms determining sex in conjunction with wing ratio variation. The inclusion of sex in polymorphic wing research is important considering the research by Brisson et al. (2007), which suggested that sexes within a single species were influenced differently by various environmental factors. It appears plausible, based on previous research and the results obtained in this study, that intrinsic and extrinsic factors are influencing wing form ratios and that these ratios may be further affected by sex ratio variation.

3.4.4 Patterns in size of insects

The differences among site in the width/length of characters suggests differential selection pressures on these populations. Based on the positive relationship between warmer temperatures and increased size in various insects groups (Colbo and Porter 1979, Colbo and Porter 1980, Nylin and Gotthard 1998) one would expect that the mean summer temperatures across the four regions (Fig. 1.1) would produce the greatest differences in average size between individuals in Fredericton, NB (warmest temperatures) and St. John's, NL (coolest temperatures), which was not observed. Although the conditions in St. John's did produce the smallest average individuals, the greatest difference between average individuals is seen between Quebec, PQ and St. John's, NL. It is possible that the high precipitation experienced in Quebec during the summer months creates favourable turf conditions, that subsequently produce large sized individuals. The nutritional qualities of turf at each sampling location was not evaluated in this study but would add additional context to future investigations of size variability

among locations. The relatively smaller size of individuals sampled from New Brunswick is not easily explained by any of the climatic variables examined in this study. However, it is possible that the number of sites sampled within this study did not provide a broad enough scale, or cover enough geographical area to allow for the detection of cross regional gradients in climate and/or size of insects. This is supported by the fact that while two sites in New Brunswick produced relatively small individuals, one separate site in New Brunswick (NB1) ranked in the top half of sites with respect to size (Brachypterous Rank=5, Macropterous Rank=2). Future work should including sampling from a greater number of more evenly spaced locations in order to develop a more complete "map" from which patterns could be more easily detected.

Inference of environment and size tradeoffs is complicated (Danks 2006) and includes many factors such as temperature, food supply (Colbo and Porter 1979, Colbo and Porter 1980), and a host of other environmental factors (Danks 2000). However, size differences were evident between populations at a regional level, suggesting some differential selective forces at this larger scale. The results presented here represent an initial step in establishing a relationship between individual size and specific habitat conditions or templets. However, it is likely that additional sampling would be required to fully extract the nature of these relationships in Quebec and Eastern Canada, which represents such a large geographic range.

There was no statistically significant relationship between growth rate in eggs and average size ranking in brachypters. Interpretation of results is limited by the fact that this experiment used the size rankings of the parents and the growth rate of their eggs to

explore this relationship. There could be several compounding factors influencing this relationship, including the possibility that adult size may influence growth rate of offspring. Further experiments using parental size and one environmental factor at a time will be necessary to elucidate this relationship between size, growth rate and temperature.

3.5 Summary

One of the primary objectives of this study was to determine whether evolutionary or plasticity adaptation had resulted in local or regionally unique sex or wing ratios. Contrary to the original prediction, sex ratio of *B. leucopterus hirtus* was consistent across collection periods and locations, indicating a stable operational sex ratio of approximately F:M=1:1.19. This decreases the usefulness of sex ratio as an indicator of habitat differentiation at either the local or regional scale. However, there were significantly more males than females found in both the Fall 2004 and Fall 2005 collection periods, suggesting female dispersal or mortality through the summer season. As hypothesized, wing form varied between locally separated populations and in the same populations over time, suggesting a response to local and regional level environmental conditions, but there was no relationship between wing form and density in this study. Although more complete studies are required to elucidate the mechanisms governing wing form, differences in wing form ratios among sites do indicate differences among sites in the environmental influences acting upon populations. The fact that more male brachypters were found compared to females suggests that the macropterous form may be useful to females in dispersal. Patterns in insect width and length suggest that conditions in St. John's, NL produce small individuals compared to most other regions

across Quebec and Atlantic Canada, with two sites in New Brunswick rating similarly to those in St. John's. This supports the hypothesis that this is likely a tradeoff for the rapid growth rate required by *B. leucopterus hirtus* in this region. There was no statistical evidence to support a correlation between growth rate and average size ranking to rearing temperature. Evidence that wing form and size vary among populations can be further explored by pest managers to predict the level of local population establishment.

Literature Cited

Abrams, P. A., O. Leimar, S. Nylin, and C. Wiklund 1996. The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. The American Naturalist 147:381-395.

Agriculture and Agri-Food Canada (AAFC). 2005. News Release: Keeping the greens green. 18 October 2005. Beloeil, Quebec, Canada.

Alcock, J. 1995. Persistent size variation in the anthophorine bee *Centris pallida* (Apidae) despite a large male mating advantage. Ecological Entomology 20:1-4.

Alcock, J. 1996. The relation between male body size, fighting, and mating success in Dawson's burrowing bee, *Amegilla dawsoni* (Apidae, Apinae, Anthophorini). Journal of Zoology 239:663-674.

Alibert, P., M. Benoit, J. L. Dommergues, and B. David. 2001. Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. Zoologica Scripta 30:299-311.

Andersen, N. M. 2000. The evolution of dispersal dimorphism and other life history traits in water striders (Hemiptera: Gerridae). Entomological Science 3:187-199.

Applebaum, S. W., and Y. Heifetz. 1999. Density-dependent physiological phase in insects. Annual Review of Entomology 44:317-341.

Arnqvist, G., and T. Mårtensson. 1998. Measurement error in geometric morphometrics: empirical strategies to access and reduce its impact on measures of shape. Acta Zoologica Academiae Scientiarum Hungaricae 44:73-76.

Atchley, E. R., and D. A. Hensleigh. 1974. The congruence of morphometric shape in relation to genetic divergence in four races of morabine grasshoppers (Orthoptera: Eumastacidae). Evolution 28:416-427.

Brisson, J. A., G. K. Davis, and D. L. Stern. 2007. Common genome-wide patterns of transcript accumulation underlying the wing polyphenism and polymorphism in the pea aphid (*Acyrthosiphon pisum*). Evolution and Development 9:338-346.

Cardini, A., A. U. Jansson, and S. Elton. 2007. A geometric morphometric approach to the study of ecogeographical and clinal variation in velvet monkeys. *Journal of Biogeography* 34:1663-1678.

Colbo, M. H., and G. N. Porter. 1979. Effects of the food supply on the life history of Simuliidae (Diptera). Canadian Journal of Zoology 57:301-306.

Colbo, M. H., and G. N. Porter. 1981. The interaction of rearing temperature and food supply on the life history of two species of Simuliidae (Diptera). Canadian Journal of Zoology 59:158-163.

Couvillon, M. J., W. O. H. Hughes, J. A. Perez-Sato, S. J. Martin, G. G. F. Roy, and F. L.W. Ratnieks. 2010. Sexual selection in honey bees: colony variation and the importance of size in male mating success. Behavioral Ecology 21:520-525.

Crnokrak, P., and D. A. Roff. 2002. Trade-offs to flight capacity in *Gryllus firmus*: the influences of whole-organism respiration rate on fitness. Journal of Evolutionary Biology 15:388-398.

Daly, H. V. 1985. Insect morphometrics. Annual Review of Entomology 30:415-438.

Danks, H. V. 1994. Diversity and integration of life-cycle controls in insects. Pages 5-40 *in* H. Danks, editor. Life cycle polymorphism: theory, evolution and ecological consequences for seasonality and diapause control. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Danks, H. V. 2000. Measuring and reporting life-cycle duration in insects and arachnids. European Journal of Entomology 97:285-303.

Danks, H. V. 2006. Key themes in the study of seasonal adaptations in Insects II. Life-cycle patterns. Applied Entomology and Zoology 41:1-13.

Danks, H. V. 2007. The elements of seasonal adaptations in insects. Canadian Entomologist 139:1-44.

de Block, M., and R. Stoks. 2007. Flight-related body morphology shapes mating success in a damselfly. Animal Behaviour 74:1093-1098.

Debat, V., A. Debelle, and I. Dworkin. 2009. Plasticity, canalization, and developmental stability of the *Drosophila* wing: joint effects of mutations and developmental temperature. Evolution 63:2864-2876.

Denno, R. F. 1994. The evolution of dispersal polymorphisms in insects: the influences of habitats, host plants and mates. Researches on Population Ecology 36:127-135.

Denno, R. F., L. W. Douglas, and D. Jacobs. 1985. Crowding and host plant nutrition: environmental determinants of wing form in *Prokelisia marginata.* Ecology 66:1588-1596.

Denno, R. F., K. L. Olmstead, and E. S. McCloud. 1989. Reproductive cost of flight capacity: a comparison of life history traits in wing dimorphic planthoppers. Ecological Entomology 14:31-44.

Denno, R. F., G. K. Roderick, K. L. Olmstead, and H. G. Dobel. 1991. Density-related migration in planthoppers (Homoptera: Delphacidae): the role of habitat persistence. The American Naturalist 138:1513-1541.

Denno, R. F., D. J. Hawthorne, B. L. Thorne, and C. Gratton. 2001. Reduced flight capacity in British Virgin Island populations of a wing-dimorphic insect: the role of habitat isolation, persistence and structure. Ecological Entomology 26:25-36.

Dujardin, J. P., T. Chavez, J. M. Moreno, M. Machine, F. Noireau, and C. J. Schofield. 1999. Comparison of Isoenzyme electrophoresis and morphometric analysis for phylogenetic reconstruction of the Rhodniini (Hemiptera: Reduviidae: Triatominae). Journal of Medical Entomology 36:653-659.

Fujisaki, K. 1993. Genetic correlation of wing polymorphism between females and males in the oriental chinch bug, *Cavelerius saccharivorus* Okajima (Heteroptera: Lygaeidae). Researches on Population Ecology 35:317-334.

Gage, M. J. G. 1995. Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella.* Proceedings of the Royal Society of London B 261:25-30.

Gatehouse, A. G. 1994. Insect migration: variability and success in a capricious environment. Researches on Population Ecology 36:165-171.

Glick, P. A. 1960. Collecting insects by plane, with special reference to dispersal of the potato leafhopper. United States Department of Agricultural Research Service Technical Bulletin 1222:1-16.

Gilburn, A. S., and T. H. Day. 1994. Evolution of female choice in seaweed flies: Fisherian and good genes mechanisms operate in different populations. Proceedings of the Royal Society B 255:159–165.

Gould, S. J., and R. F. Johnston. 1972. Geographic variation. Annual Review of Ecology and Systematics 3:457-498.

Hamilton, W. D., and R. M. May. 1977. Dispersal in stable habitats. Nature 269:578-581.

Harada, T., S. Nitta, and K. Ito. 2005. Photoperiodism changes according to global warming in wing-form determination and diapause induction of a water strider,

Aquarius paludum (Heteroptera: Gerridae). Applied Entomology and Zoology 40:461-466.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annual Review of Entomology 11:95-118.

Hernández-Ortiz, V., J. A. Gómez-Anaya, A. Sánchez, B. A. McPheron, and M. Aluja. 2004. Morphometric analysis of Mexican and South American populations of the *Anastrepha fraterculus* complex (Diptera:Tephritidae) and recognition of a distinct Mexican morphotype. Bulletin of Entomological Research 94:487-499.

Herrmann, D. L., A. E. Ko, S. Bhatt, J. E. Jannot, and S. A. Juliano. 2010. Geographic variation in size and oviposition depths of *Romalea microptera* (Orthoptera: Acrididae) is associated with different soil conditions. Annals of the Entomological Society of America 103:227-235.

Hespenheide, H. A. 1973. Ecological inferences from morphological data. Annual Review of Ecology and Systematics 4:213-229.

Honěk, A. 1993. Intra-specific variation in body size and fecundity in insects: a general relationship. Oikos 66:483-492.

Horton, D. R., and T. M. Lewis. 2000. Seasonal Distribution of *Anthocoris* spp. and *Deraeocoris brevis* (Heteroptera: Anthocoridae, Miridae) in Orchard and Non-Orchard Habitats of Central Washington. Annals of the Entomological Society of America 93:476-485

Horton, D. R., T. M. Lewis, T. Hinojosa, and D. A. Broers. 1998. Photoperiod and reproductive diapause in the predatory bugs *Anthocorsis tometosus*, *A. antevolens*, and *Deraeocoris brevis* (Heteroptera: Anthocoridae, Miridae) with information on overwintering sex ratios. Annals of the Entomological Society of America 91:81-86.

Jannot, J. E., A. E. Ko, D. L. Herrmann, L. Skinner, E. Butzen, O. Akman, and S. Juliano. 2009. Density-dependent polyphenism and geographic variation in size among two populations of lubber grasshoppers (*Romalea microptera*). Ecological Entomology 34:644-651.

Julliard, R. 2000. Sex-specific dispersal in spatially varying environments leads to a habitat-dependent evolutionarily stable offspring sex ratio. Behavioral Ecology 11:421-428.

Kaitala, A., and H. Dingle. 1992. Spatial and temporal variation in wing dimorphism of California populations of the waterstrider *Aquarius remigis* (Heteroptera: Gerridae). Annals of the Entomological Society of America 85:590-595.

Kawecki, T. J. 1993. Age and size at maturity in a patchy environment: fitness maximization versus evolutionary stability. Oikos 66:309-317.

Langellotto, G. A., and R. F. Denno. 2001. Benefits of dispersal in patchy environments: mate location by males of a wing-dimorphic insect. Ecology 82:1870-1878.

Langellotto, G. A., R. F. Denno, and J. R. Ott. 2000. A trade-off between flight capability and reproduction in males of a wing-dimorphic insect. Ecology 81:865-875.

Leonard, D. E. 1966. Biosystematics of the *leucopterus* complex of the genus *Blissus* (Hemiptera: Lygaeidae). Bulletin - Connecticut Agricultural Experiment Station 677:1-47.

Maderbacher, M., C. Baker, L. Herler, L. Makasa, and C. Sturmbauer. 2008. Assessment of traditional versus geometric morphometrics for discriminating populations of the *Tropheus moorii* species complex (Teleostei: Cichilidae), a Lake Tanganyika model for allopatric speciation. Journal Zoological Systematics and Evolution Research 46:153-161.

Madjdzadeh, S. M., and M. Mehrparvar. 2009. Morphological discrimination of geographical populations of *Macrosiphoniella sanborni* (Gillette, 1908) (Hem.: Aphididae) in Iran. North-Western Journal of Zoology 5:338-348.

Mailloux G., and H. T. Streu. 1981. Population biology of the hairy chinch bug (*Blissus leucopterus hirtus*, Montandon: Hemiptera: Lygaeidae). Annals of the Entomological Society of Quebec 26:51-90.

Milenković, M., V. J. Šipeteć, J. Blagojević, S. Tatović, and M. Vujošević. 2010. Skull variation in Dinaric-Balkan and Carpathian gray wolf populations revealed by geometric morphometric approaches. Journal of Mammology 91:376-386.

Nylin, S. 1994. Seasonal plasticity and life cycle adaptations in butterflies. Pages 41-67 *in* H. Danks, editor. Life cycle polymorphism: theory, evolution and ecological consequences for seasonality and diapause control. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Nylin, S., and K. Gotthard. 1998. Plasticity in life history traits. Annual Review of Entomology 43:63-83.

Pen, I., and F. J. Weissing. 2002. Optimal sex allocation: steps towards a mechanistic theory. Pages 26-47 *in* I. C. W. Hardy, editor. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge, UK.

Pfenning, B., S. Gerstner, and H. J. Poethke. 2008. Alternative life histories in the water strider *Gerris lacustis*: time constraint on wing morph and voltinism. Entomologia Experimentalis et Applicata 129:235-242.

Potter, D. A. 1998. Destructive turfgrass insects: biology, diagnosis and control. Ann Arbor Press, Chelsea, Michigan, USA.

Roff, D. A. 1986. The evolution of wing dimorphism in insects. Evolution 40:1009-1020.

Roff, D. A. 1990. The evolution of flightlessness in insects. Ecological Monographs 60:389-421.

Roff, D. A. 1994. Habitat persistence and the evolution of wing dimorphism in insects. The American Naturalist 144:772-798.

Roff, D. A., and D. J. Fairborn. 1991. Wing dimorphism and the evolution of migratory polymorphisms among the Insecta. American Zoologist 31:243-251.

Sasaki, R., F. Nakasuji, and K. Fujisaki. 2002. Environmental factors determining wing form in the lygaeid bug, *Dimophopterus japonicas* (Heteroptera: Lygaeidae). Applied Entomological Zoology 37:329-333.

Sauer, D., and D. Feir. 1973. Studies on natural populations of *Oncopeltus fasciatus* (Dallas), the large milkweed bugs. American Midland Naturalist 90:13-37.

Schilthuizen, M., A. van Til, M. Salverda, T. S. Liew, T. S. James, B. bin Elahan, and J. J. Vermeulen. 2006. Microgeographic evolution of snail shell shape and predator behaviour. Evolution 60:1851-1858.

Scriber, J. M. 1994. Climatic legacies and sex chromosomes: latitudinal patterns of voltinism, diapause, size, and host-plant selection in two species of swallowtail butterflies at their hybrid zone. Pages 133-171 *in* H. Danks, editor. Life cycle polymorphism: theory, evolution and ecological consequences for seasonality and diapause control. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Seger, J., and J. W. Stubblefield. 2002. Models of sex ratio evolution. Pages 218–234 *in* I. C. W. Hardy, editor. Sex ratios: concepts and research methods. Cambridge University Press, Cambridge, UK.

Shama, L. N. S., and C. T. Robinson. 2009. Microgeographic life history variation in an alpine caddisfly: plasticity in response to seasonal time constraints. Freshwater Biology 54:150-164.

Sibley, R. M., and D. Atkinson. 1994. How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology* 8:486-493.

Slaney, D. P., and D. Blair. 2000. Molecules and morphology are concordant in discriminating among populations of cave cockroaches in the Genus *Paratemnopteryx* Saussure (Blattodea: Blattellidae). Annals of the Entomological Society of America 93:398-404.

Sluss, T. P., H. M. Graham, and E. P. Sluss. 1982. Morphometric, allozyme and hybridization comparisons of four *Lygus* species (Hemiptera: Miridae). Annals of the Entomological Society of America 75:448-456.

Socha, R., and J. Šula. 2006. Flight muscles polymorphism in a flightless bug, *Pyrrhocoris apterus* (L.): Developmental pattern, biochemical profile and endocrine control. Journal of Insect Physiology 52:231-239.

Solbreck, C. 1978. Migration, diapause, and direct development as alternative life strategies in a seed bug, *Neacoryphus bicrucis*. Pages 195-217 *in* H. Dingle, editor. Evolution of insect migration and diapause. Springer-Verlag, New York, New York, USA.

Southwood, T. R. E. 1962. Migration of terrestrial arthropods in relation to habitat. Biological Review 37:171-214.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology 46:336-365.

Statzner, B., A. G. Hildrew, and V. H. Resh. 2001. Species traits and environmental constraints: entomological research and the history of ecological theory. Annual Review of Entomology 46:291-316.

Sweet, M. H. 1963. A new species of *Ligyrocoris* Stal with a key to the northeastern species (Hemiptera: Lygaeidae). Psyche 70:17-21.

Tanaka, S. 1976. Allocation of resources to egg production and flight muscle development in a wing dimorphic cricket, *Modicogryllus confirmatus*. Journal of Insect Physiology 39:493-498.

Tuxen, S. L. 1967. The Entomologist, J. C. Fabricius. Annual Review of Entomology 12:1-15.

Uller, T., P. Ido, E. Wapstra, L. W. Beukeboom, and J. Komdeur. 2007. The evolution of sex ratios and sex-determining systems. Trends in Ecology and Evolution 22:292-297.

Vittum, P., M. Villani, and H. Tashiro. 1999. Turfgrass insects of the United States and Canada, 2nd ed. Cornell University Press, Ithaca, New York, USA.

Wei, Y.J. 2008. Sex ratio of *Nysius huttoni* White (Hemiptera: Lygaeidae) in field and laboratory populations. New Zealand Journal of Zoology 35:19-28.

Wellwood, A., G. Nickerson, and J. Wetmore. 2002. Hairy chinch bug survey, demonstration and monitoring in New Brunswick, 2002. New Brunswick Department of Agriculture, Fisheries and Aquaculture and New Brunswick Horticultural Trades Association, Fredericton, New Brunswick, Canada.

Werner, E. E., and B. R. Anholt. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. The American Naturalist 142:242-272.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press, New York, New York, USA.

Wilfert, L., M. Kaib, W. Durka, and R. Brandl. 2006. Differentiation between populations of a termite in eastern Africa: implications for biogeography. Journal of Biogeography 33:1993-2000.

Zera, A. J. 1984. Differences in survivorship, development rate and fertility between the long-winged and wingless morphs of the waterstrider, *Limnoporus canaliculatus*. Evolution 38:1023–1032.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42:207-30.

Zera, A. J., and S. Mole. 1994. The physiological costs of flight capability in wing-dimorphic crickets. Researches on Population Ecology 36:151-156.

Zera, A. J., and K. C. Tiebel. 1988. Brachypterizing effect of group rearing, juvenile hormone III and methoprene in the wing-dimorphic cricket, *Gryllus rubens*. Journal of insect Physiology 34:489-498.

Zhang, S. M. 1985. Economic insect fauna of China, Fascicle 31, Hemiptera (1). Science Press, Beijing, China.

Chapter 4: Host Choice Behaviour of *Blissus leucopterus hirtus* Montandon (Hemiptera: Blissidae) in Relation to Geographic Distribution, Insect Age and Insect Group Size

4.1 Introduction

4.1.1 Host Choice Behaviour

Insect host choice is a term that is used to describe preference in herbivorous insects toward a given plant or group of plants for purposes that may include oviposition, feeding, basking or predator avoidance. Insect feeding patterns within a single species can vary across geographical areas as a result of resource availability and quality (Diamond and Kingsolver 2010, Wennström et al. 2010). Changes in host choice preference may result in local or regional level adaptation in several physiological systems, including communications, as in the case of the *Enchenopa binotata* complex (Hemiptera: Membracidae), where vibrational mating signals are transmitted through plant stems (Cocroft et al. 2010). Other aspects of life history potentially affected include mate choice (Abrahamson et al. 2001, Vanbergen et al. 2003, Görür 2005, Nosil et al. 2005, Görür et al. 2007), fecundity (Awmack and Leather 2002), morphology (Vanbergen et al. 2003) and developmental time and growth rate (Grossmueller and Lederhouse 1985, Nylin and Gotthard 1998). For example, local differences in host choice may reflect subtle variation in the relative quality of potential hosts (Strauss and

Karban 1998, Singer et al. 2002). Differences in plant quality may in turn be a reflection of plant genetics or more extrinsic factors such as rainfall, soil conditions, sun exposure, plant competition, or reduced plant defenses due to previous attack (Carrière and Roitberg 1995, Karban and Baldwin 1997). In addition to direct nutritional quality of plants, other factors, including the local presence of predators, entomopathogens (such as *Beauveria bassiana* Bals.) and parasitoids may also influence local host choice (Dicke 1994). The specificity of the relationship between an insect herbivore and its host plant may be strong enough to promote reproductive population isolation and subsequent phenotypic and genetic differentiation (Futuyma and Peterson 1985, Berlocher and Feder 2002, Grace et al. 2010). Factors influencing host choice behaviour in *B. leucopterus hirtus* at a local and regional level, where varying environmental (extrinsic) and physiological (intrinsic) factors such as age and population size exist, may influence host choice spatially and/or temporally.

4.1.2 Host Choice across Local and Regional Population Spatial Scales

Research on various insect species shows host choice and degree of host specificity varies among geographically separated populations (Papaj 1986, Prokopy et al. 1988, Caillaud and Via 2000, Funk and Bernays 2001, Gotthard et al. 2003). This specialization can lead to divergent host plant adaptation and eventual reproductive isolation, examples of which are already described in several insect orders including Orthoptera (Grace et al. 2010) and Coleoptera (Egan et al. 2008). Shifts in host choice can also lead to adjustments in life history to optimize life strategy in novel environments (Vanbergen et al. 2003). However, there are exceptions; Wehling and Thompson (1997)

found that oviposition preference hierarchy was preserved in a widespread polyphagous butterfly despite differences in host plant availability among populations of the insect across its range. While variation in host preference is commonly observed and studied at a regional level, host plant quality, a potential factor in host preference, can vary at a small scale due to mineral and microclimatic differences that result in differences in soil quality, nutritional quality, plant and insect phenology, enemy presence and other environmentally dependent factors (Bernays and Graham 1988, Awmack and Leather 2002, Vanbergen et al. 2003, Diamond and Kingsolver 2010). Over a large geographic region such as Quebec and Eastern Canada, where differences in climate are evident, one could hypothesize that variation in host choice behaviour exists and that it may be an important variable in discrimination of populations. Differences among populations in host choice behaviour is a third marker of variation in life strategy, in addition to phenology (Ch. 2) and morphology (Ch. 3) that may occur as a result of specific environmental pressures (Scriber 1994), and adds insight into the effect of geographical and climatic variation in insect life history. Using Southwood's habitat templet model (Southwood 1977) as a foundation to explain population disparity, research into the variables driving host choice at the population scale of *B. leucopterus hirtus* should facilitate prediction of attributes that make an area favourable to colonization, and from this, provide information to pest managers who are making recommendations on turf species and their management to homeowners across this pest range. For example, better insight may be provides on what types of species/cultivars to plant, and where the insects are most liekly to be found at certain times of the year,

4.1.3 Intrinsic Factors Influencing Host Choice

4.1.3.1 Age of insect

In addition to the environmental determinants, there are several intrinsic life history variables that can result in host choice differences among populations of an insect species. Mode of feeding, food choice and rate of feeding can differ among instars. For example, the lack of mobility in some early instars limits feeding choices in some insect species to hosts selected by the female during oviposition and, therefore, limits host choice (Wennström et al. 2010). As insects mature, instars feeding on different plants during specific periods of their ontogeny may reflect differences in nutritional requirements (Slansky and Scriber 1985). For example, feeding trials with caterpillars of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: *Lymantriidae*) showed that as these insects developed from 3rd to 6th instar, they exhibited increased preference for diet cubes with high lipid concentration in conjunction with decreased preference for diet with high protein concentration. This likely reflected changing nutritional needs (Stockoff 1993). Feeding trials employing insects of only one instar may, therefore, not indicate preferences at another stage of their ontogeny or that of potential host communities. A strong indication that host preference may vary seasonally with *B. leucopterus hirtus* is that the common chinch bug, *B. leucopterus leucopterus* Say (Hemiptera Blissidae) has been observed in the field to move between crop hosts including small grains, corn and sorghum during the spring and summer months (Headlee and Walker 1913, Packard 1937, Lamp and Holtzer 1980). This may indicate changes in nutritional value of crops or physiological change in the insect over time, which was assist pest managers in

determining when certain plants or grass types may be more susceptible to damage. Although physiological changes may be responsible for, or accompany changes, in feeding behaviour in some insects, the specific labial tip sensory sensilla do not appear to differ and thus are not a factor in age-specific host selection of *B. leucopterus hirtus* (Baker et al. 2008).

4.1.3.2 Insect density and group dynamics

The life history strategy of an insect can include a density dependent response. In certain cases, the result is physiological or morphological change; locusts (Pener and Yerushalmi 1998) and aphids (Braendle et al. 2006) are classic examples. One manifestation of the density response includes host choice behaviour. Some of the effects of high density are resource use efficiency, protection from natural enemies while feeding and indirect benefits of mate finding and protection from environmental conditions (Gullan and Cranston 2005). As an example, caterpillars of *Chlosyne janais* Drury (Lepidoptera: Nymphalidae) and *Chlosyne poecile* Felder (Lepidoptera: Nymphalidae) have all shown increased ability to penetrate thicker leaf tissues when feeding in large groups (Denno and Benrey 1997, Inouye and Johnson 2005). Similar advantages have been recorded for certain aphids, because of the ability of large aggregations to redirect the flow of nutrients to a wounded area in host trees (Way and Cammell 1970). Large groups may therefore facilitate feeding on hosts that would otherwise be inaccessible.

In contrast, high densities of insects may deter an insect from choosing a particular host. Costs of feeding aggregations include competition, transmission of diseases and/or

parasitoids, deteriorating environmental conditions and increased conspicuousness. An example of egg density deterring host selection is found in *Pieris brassicae* L. (Lepidoptera: Pieridae), an insect that will oviposit on a less favourable plant when egg overloading is observed on the preferred species (Rothschild and Schoonhoven 1977). Knowing whether host preference of *B. leucopterus hirtus* is affected by population density should provide further understanding of the population dynamics of this species and its potential impact as a pest at both low and high densities.

4.1.4 Host Choice Behaviour in *Blissus leucopterus hirtus*

Blissus leucopterus hirtus is a widespread turfgrass and crop pest in Quebec and Atlantic Canada and the northeastern United States (Leonard 1966, Vittum et al. 1999, Potter 1998). In Canada, *B. leucopterus hirtus* is a destructive turf pest in Ontario, Quebec and the Atlantic provinces. Within the United States, its range extends as far west as East Minnesota and south into North Virginia. In the last decade *B. leucopterus hirtus* has been recognized as a pest of turfgrass in Newfoundland, an island off the east coast of Canada. The species was first recorded in Newfoundland in 1949 by Brown (Brown 1949), the year the province joined Canadian Confederation. Whether its presence on the island is the result of an introduction from the mainland of North America, or due to better faunal surveys conducted by the Government of Canada is not certain.

Although host species specificity exists in phytophagous insects (Jaenike 1990, Bernays and Chapman 1994, Schoonhoven et al. 1998), *B. leucopterus hirtus* and other closely related species within *Blissidae* feed on several monocots, and *B. leucopterus*

leucopterus is often associated with damage to plants other than turfgrasses, including corn and sorghum (Ahmad et al. 1984, Ramoska and Todd 1985). General research has been conducted on the survival and fecundity of *Blissus leucopterus leucopterus* on various corn and sorghum lines (Mize and Wilde 1986a, 1986b) and on host preference among turfgrasses in the western chinch bug, *Blissus occiduus* Barber (Hemiptera: Blissidae) (Eickhoff 2002, Eickhoff et al. 2006). The southern chinch bug, *Blissus insularis* Barber (Hemiptera: Blissidae) has also shown feeding flexibility and has even overcome avoidance to previously resistant cultivars. Specifically, the insect was able to overcome resistance of a new cultivar of St. Augustinegrass 'Floratum' within twelve years of the turfgrass' release, as selective pressures increased both the longevity of females and the weekly oviposition rate in populations on this host (Busey and Center 1987, Busey 1990). Since *B. leucopterus hirtus* is closely related to these species, these observations suggest that *B. leucopterus hirtus* may have turf-specific polyphagous potential (Eickhoff et al. 2004). This flexibility may even include species not yet recorded as hosts.

In fact, at present, *B. leucopterus hirtus* has been recorded feeding on cool-season turfgrasses including creeping bentgrass, Kentucky bluegrass, perennial ryegrass, fine-leaf fescues, and timothy grass, and warmer season grasses such as zoysiagrass (Vittum et al. 1999). However, laboratory rearing is often achieved through feeding on corn because it is easily grown and grows rapidly (Baker et al. 1981). Much attention has been given in recent years to *B. leucopterus hirtus'* interaction with grasses that either naturally contain, or have been inoculated with endophytic fungi (a fungus living symbiotically within a

grass or plant). These endophytes have potential to promote avoidance or toxicity in phytophagous insects (Saha et al. 1987, Breen 1994, Clay 1996, Carrière et al. 1998, Richmond and Shetlar 2000, Bourassa et al. 2007). There is evidence that nymphs of *B. leucopterus hirtus* have the capacity to discriminate between endophyte and non-endophyte inoculated grass cultivars at a micro-geographic scale (Mathias et al. 1990, Carrière et al. 1998), making endophyte presence/absence an important factor in the characterization of potential hosts and thus host choice.

Although there has been some research on host choice in *B. leucopterus hirtus*, there has been little investigation of possible variation in host choice among populations. Confirming the existence of differences in host choice among populations is the first step in establishing host choice as a life history characteristic subject to change as a result of environmental conditions, for it is well established that environmental conditions have influence over the fauna within an areas. Eventually elucidating the specific factors responsible for these difference in host choice should allow turf scientists and managers to better predict potential areas of infestation.

4.1.5 Research Objectives

This study characterized the host choice preference of *B. leucopterus hirtus* to identify differences in these characteristics at the local and regional scale. Based on current knowledge of *B. leucopterus hirtus* and the habitat template theory, hypotheses are as follows:

1) Based on evidence from closely related species *(B. leucopterus leucopterus)* and preliminary observations in other studies, *B. leucopterus hirtus* display differences in plant host preference among a) locally separated populations in St. John's, NL and/or b) regionally separated populations of *B. leucopterus hirtus* in Quebec and Atlantic Canada.

2) Based on the changing needs on an insect through its ontogeny, related both to shelter and food, early instars of *B. leucopterus hirtus* display preference for soft, fine "feeding" grasses, which later instars show preference for coarser, sheltering grasses.

3) Based on previous observation that individuals of *B. leucopterus hirtus* have the capacity to aggregate toward later instars, host preference is influenced by aggregation in the later instars. Therefore, individuals are more randomly distributed among grass tufts while groups may cluster together on various plants. Host preference in *Blissus leucopterus hirtus* is affected by whether an individual insect is feeding in isolation or in a group.

4.2 Materials and Methods

The above research questions were addressed through a series of laboratory host-plant choice tests. Experimental 'arenas' in which *B. leucopterus hirtus* were allowed to make a choice among either twelve or four varieties of grass were used. The null hypothesis tested was that there would be no significant difference in choice for any treatment scenario. These experiments were based on host choice (i.e. an insect was

observed on or near a plant) and were not designed to verify feeding or oviposition choice, since neither puncture wounds nor egg counts were examined. Comparisons between locally and regionally separated populations were based on the collection of live adults from 13 locations in Quebec and Atlantic Canada. Local populations were sampled from sites within a few kilometers of each other, with the exception of New Brunswick, where based on local recommendation, two populations were sampled in Fredericton, NB, and one 82 km away, near Saint John, NB. 'Regional areas' were defined by provincial boundaries, with province names serving as identifiers for these site groupings.

4.2.1 Experimental Setup

In May 2008, *B. leucopterus hirtus* over wintering adults were collected from thirteen sites in Quebec and Atlantic Canada: three in Quebec City (PQ), two in Fredericton (NB), one in Saint John (NB), three in the Annapolis Valley (NS) and four in St. John's, (NL) (Fig. 4.1). The four populations selected in St. John's in 2008 were independent of the 10 sites sampled from 2004-2005. For time and efficiency, adults were collected using a combination of hand sampling with tweezers and vacuum sampling in areas identified by local entomologists as inhabited by *B. leucopterus hirtus* populations (Appendix C). Over the 14-day collection trip, captured individuals were stored in plastic containers with grass and plant debris from the local collection area and kept in a cooler above ice packs that were refrozen daily. Upon arrival in St. John's, the adult bugs were transferred into site-specific large sized plastic Ziploc® Snap 'n Seal containers, with each container considered an individual colony.

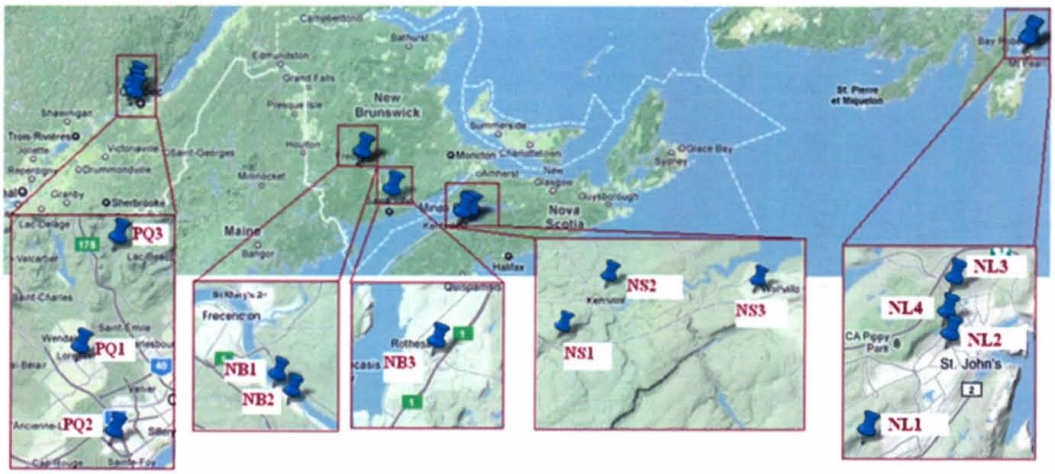

FIGURE 4.1. Location of twelve study sites across Atlantic Canada. Sites are labeled in enlarged map sections.

The containers were modified by replacing the circular centre section of the lid (d=10 cm) with a 0.2 mm mesh that promoted airflow but prevented the bugs from escaping. Plant material and debris in the containers were removed and all colonies were fed stem sections from green house reared *Zea mays* var. polka (Veseys Seed Company, Charlottetown, PEI, Canada) between 3 leaf and 4 leaf stage. The colonies were placed into a Conviron® growth chamber maintained at 20°C±1°C, with a 16:8 hour photoperiod at 100% relative humidity. Several individuals from each colony were killed, preserved in 70% ethanol and sent to Agriculture and Agri-Food Canada in Ottawa, Ontario, Canada for identification at the Canadian National Collection. All samples submitted were confirmed as *B. leucopterus hirtus*. Individuals were allowed to mate and produce eggs, which were collected and transferred to separate containers. All eggs and nymphs were reared under identical conditions to their parents until they were required for experiments.

Experiment 1: Initial comparison and selection of cultivars: Following the protocols of Eickhoff et al. (2006), a preliminary evaluation of host preference with 12 grass cultivars used commercially in Newfoundland was carried out in the laboratory (Table 4.1). All grass varieties were grown from seed provided by Pickseed Canada Inc. (Lindsay, ON, Canada) with the exception of the timothy seed, which was purchased at a gardening store in St. John's. All of the grasses were labeled endophyte positive or endophyte free. However, as a second precaution, each cultivar was tested at 6 weeks of age for the presence of endophytes using a *Neotyphodium* Field Tiller Phytoscreen Immunoblot Kit (Agrinostics, Ltd. Watkinsville, GA, USA). All grasses tested positive or negative for endophyte as labeled on the original package. With minor modifications, the experiments followed the procedures of Eickhoff et al. (2006). Experiments began when grass was six weeks old. Experimental arenas were created in medium sized Ziploc® Twist 'n Lock containers, in which the lids were modified by replacing the circular centre section of each lid (d=5 cm) with a 0.2 mm mesh that promoted airflow but prevented escape.

TABLE 4.1. Twelve cultivars initially investigated for attractiveness to *B. leucopterus hirtus*.

Grass Type	Cultivar	Latin name	Endophyte +
Rye	Common Rye	*Lolium perenne*	No
	Fiesta	*Lolium perenne*	Yes
Red fescue	Common Red	*Festuca rubra*	No
	Jasper	*Festuca rubra*	Yes
Tall fescue	Fawn	*Festuca arundinacae*	No
	Team Jr.	*Festuca arundinacae*	Yes
Timothy	*None specified*	*Phleum pratense*	No
Sheep fescue	Azay	*Festuca ovina*	No
Creeping bent	*None specified*	*Agrostis palustris*	No
Kentucky	Touchdown	*Poa pratensis*	No
bluegrass	Mercury	*Poa pratensis*	No
	America	*Poa pratensis*	No

Twenty-four hours prior to each experiment, grass heights were standardized to four cm by trimming each tiller with garden scissors. The next day, one tiller was randomly selected from each cultivar bed and its roots gently washed with tap water. The roots of the tiller were placed into water filled florists' hydro-pics so that the tiller was exposed above the hydro-pic rim. Tillers were secured into place by stretching Parafilm® over the hydro-pic opening. The cultivars were placed in an arena with each hydro-pic containing one tiller of one grass type and placed into one of 12 evenly spaced holes around the bottom of the container (Fig. 4.2). Nine arena replications in which the placement of the tillers was randomized were created for this experiment, with containers serving as blocs. Twenty five *B. leucopterus hirtus* individuals in 4th and 5th instar were introduced into the centre of each arena (Fig. 4.2). The individuals were of mixed gender from NL2, a population with a high number of available individuals. The number of

individuals found on or within 1 cm of each tiller was recorded at 1, 2, 4, 8, 24, 48 and 72 hours. The 1 cm radius was chosen because although extreme care was taken when removing the arenas from the growth chamber, the movements and change in light conditions may have caused some disturbance and movement among the insects. The 1 cm radius typically included the surface directly below each tiller, on the wax surface of the Parafilm®.

FIGURE 4.2. Experimental set up in which 25 individuals of *B. leucopterus hirtus* were allowed to choose between 12 different cultivars of common grasses.

Based on the results of this experiment, four of the initial 12 cultivars, which differed in both attractiveness to the NL2 population and in various physical characteristics (Table 4.2), were used in *Experiments 2-5*. To reduce the number of potential variables, a criterion for the final four selected grasses were that they were free of endophyte. All four of the final cultivars tested negative for endophytes. For the four grasses, timothy was chosen because of its unique texture and appeal in *Experiment 1*

(see results below). The cultivar Mercury was chosen because it was the most frequently selected of the Kentucky bluegrasses during *Experiment 1* and because Kentucky bluegrass is considered a preferred host for *B. leucopterus hirtus*, and unlike timothy, has a soft, fine texture. The sheep fescue Azay was chosen because it has unique architectural characteristics (Table 4.2) and of its initial high favourability to the insects during the 72 hour trial. Finally, the tall fescue Fawn was added to the final four grasses because of its similar physical characteristics to timothy, but with lesser apparent appeal to the NL2 population.

TABLE 4.2. General description of four grasses used in host choice experiments

Grass	**Latin name**	**General type***	**Leaf Texture****	**Blade width*****
Timothy	*Phleum pratense*	Bunch	Coarse	Wide
Kentucky bluegrass (Mercury)	*Poa pratensis*	Sod forming	Fine	Medium
Tall fescue (Fawn)	*Festuca arundinacae*	Bunch	Coarse	Wide
Sheep fescue (Azay)	*Festuca ovina*	Bunch	Very Fine	Narrow

* Bunch grasses form bunches or clumps and spread only by seed. Sod forming grasses will form rhizomes that bind together the soil.

** Texture was assigned based on technical data provided by Pickseed Canada Inc. (Lindsay, ON, Canada) in combination with subjective assessment by the researchers.

***Blade width was assigned based on technical data provided by Pickseed Canada Inc. (Lindsay, ON, Canada) and an average width of 20 randomly selected grass blades (Narrow=<1 mm, Medium=1-5 mm, Wide=>5 mm.

Following *Experiment 1*, four additional experiments were conducted, the variables of which are summarized in Table 4.3. In comparisons of host choice among local and regional populations, early instars, which are more likely to be feeding, were used. The preferences of early instars are then compared to later instars in a comparison

of feeding preference by age. Finally, the effect of group size is compared by using later instars (4 and 5) because of the tendency of these older insects to aggregate when in groups.

TABLE 4.3. Summary of variables in *Experiments 2-5*, indicating the variable examined, instar, the number of collection sites included, the number of replications, and the number of *B. leucopterus hirtus* used per replication.

Exp.	Variable	Instar	Sites	Block/Replications	Insects per replication
2	Local Population (St. John's, NL)Regional Populations	1	4	5	10
3	Regional Populations	1	4	5	10
4	Age of HCB	1 and 5	1	Each instar: 5	10
5	Group size (1 vs 10)	5	4	Group: 5 Individual: 13-25	Group: 10 Individual: 1

Experiments 2-5 were conducted when grass was just over six weeks old. As with the previous trial, twenty-four hours prior to each experiment, grass heights were standardized to four cm by trimming each tiller with garden scissors. The next day, groups of 5 tillers were randomly selected from each cultivar bed and their roots gently washed with tap water. The roots of grouped tillers were placed into water filled florists' hydro-pics so that the tiller was exposed above the hydro-pic rim. Tillers were secured into place by stretching Parafilm® over the hydro-pic opening (Fig. 4.3).

Experimental arenas were created in 'medium' sized Ziploc® Twist 'n Lock containers, in which the lids were modified by replacing the circular centre section of each lid (d=5 cm) with a 0.2 mm mesh that promoted airflow but prevented escape. Four grass cultivars were placed in an arena with each hydro-pic containing 5 tillers of one grass type and placed into one of four evenly spaced holes around the bottom of the container (Fig. 4.3).

FIGURE 4.3. Experimental set-up to determine host choice in *B. leucopterus hirtus*.

Experiment 2: Comparison of host preference in locally separated populations: For each of four locally separated populations in St. John's, NL, ten 1st instar nymphs of *B. leucopterus hirtus* were placed into the centre of an arena. There were 5 replications for a total of 200 insects in the experiment (5 replications x 10 insects x 4 local populations). The arenas were placed into a Conviron® growth chamber maintained at 20°C±1°C, with a 16:8 hour photoperiod at 100% relative humidity. After 72 hours, nymphs observed within 1 cm of a tiller group were counted as having chosen that cultivar. Individuals outside of that radius were not counted.

Experiment 3: Comparisons of host preference in regionally separated populations: Within each of the four regions sampled (Quebec, New Brunswick, Nova Scotia, Newfoundland), the site with the highest numbers of collected individuals (PQ3 NS2,

NB2, NL2) (to ensure sufficient numbers) was selected for regional comparisons among populations. For each population, ten 1st instar nymphs were placed into the centre of an arena. There were 5 replications for a total of 200 insects in the experiment (5 replications x 10 insects x 4 regional populations). The arenas were placed into a Conviron® growth chamber maintained at 20°C±1°C, with a 16:8 hour photoperiod at 100% relative humidity. After 72 hours, nymphs observed within 1 cm of a tiller group were counted as having chosen that cultivar. Individuals outside of that radius were not counted.

Experiment 4: Comparison of host preference between 1st and 5th instar nymphs: For each of these two age categories, ten nymphs from a densely populated site in the Annapolis Valley of Nova Scotia were placed into the centre of an arena. This procedure was replicated 5 times for both 1st and 5th instar nymphs for a total of 100 insects (5 replications x 10 insects x 2 age groups). For unknown reasons, initial mortality for 5th instar were high, so an additional 54 5th instars were placed into new arenas, distributed according to the procedures outlined above. The arenas were placed into a Conviron® growth chamber maintained at 20°C±1°C, with a 16:8 hour photoperiod at 100% relative humidity. After 72 hours, nymphs observed within 1 cm of a tiller group were counted as having chosen that cultivar. Individuals outside of that radius were not counted. The results of these trials involving 5th instar insects were also used in Experiment 5.

Experiment 5: Comparison of host preference between one individual and groups of 10 individuals: Within each of the four regions sampled (Quebec, New Brunswick, Nova Scotia, Newfoundland*), the site with the highest population numbers was selected to test

the effect of density on host choice preference. Nymphs in the 5th instar were placed into an arena either individually or in groups of 10. For the groups of 10 nymphs, 5 replications were used for each region, and these trials were done as part of Experiment 4. For bugs placed individually, the number of replications varied from 13-42, depending on availability of nymphs from each site. After 72 hours, nymphs observed within 1 cm of a tiller group were counted as having chosen that cultivar. Individuals outside of that radius were not counted. *Because of limited availability of insects at this point, the results of groups text on 5th instars from experiments 2 and 3 were incorporating into this data analysis to compensate for low number in Newfoundland.

4.2.2 Data Analysis

Only outcomes in which individual insects "chose" a cultivar (were found within 1 cm of a tiller group) were included in all analyses. This is because some individuals dies or became immobilized by petroleum jelly used in the experiments to keep insects from escaping. For all these experiments, each insect was considered individually with regard to choice made. In *Experiment 1*, twelve cultivars were evaluated for their attractiveness to *B. leucopterus hirtus* by comparing the mean number of *B. leucopterus hirtus* among tillers at 1, 2, 8, 24, 36 and 72 hours using a Kruscal-Wallis test. The results at 72 hours were further analyzed with a post hoc Wilcoxin pair comparison using Holm's correction.

For *Experiments 2-5*, in which only four grass tillers were used, a binary form of analysis was chosen because of the lower number of possible choices, combined with the

robust quality of a binary error structure (Sarkar and Midi 2010). Replicates were pooled and if a bug chose cultivar 1, the results for that bug would be 1/1 successes for cultivar 1 and 0/1 successes for all other cultivars. This binomial approach contrasts with the similar experiment by Eickhoff et al. (2006) in which a normal error structure was employed. In the present experiments, data were analyzed using the generalized linear model. The specific model used was a binary logistic regression routine with factors (e.g. grass type) assigned numbers as 'dummy variables'. The binary model produces results in the form of odds ratios, which can then be converted into probabilities using the formula:

$$P=odds/(1+odds)$$

To facilitate interpretation, the odds of a given bug choosing a particular cultivar compared to a chosen 'base cultivar' was then converted to the probability of finding a bug on a particular cultivar. The base cultivar is one of the grass types against which all others are compared. In this case, the arbitrarily selected base cultivar was the sheep fescue. (Subsequent substitution on alternate bases produced identical conclusions). This model allowed comparison of the odds of an insect from one origin choosing a certain type of grass versus the odds of an insect from a separate origin choosing the same type of grass. Mosaic plots express these probabilities and were created using JMP® 7.0.1. JMP® 7.0.1 (SAS Institute Inc., Cary, NC, USA).

4.3 Results

4.3.1 Experiment 1: Initial Comparison and Selection of Cultivars:

Data was not normal so non-parametric methods were used to assess differences among host choice. Based on nine replications, the mean number of *B. leucopterus hirtus* individual 4^{th} and 5^{th} instars on each tiller varied significantly among cultivars at 1 hour (KWT p=0.012), 8hrs (KWT p=0.0490) 24 hours (KWT p=0.0015), 48 hours (KWT p=0.0006), and 72 hours (KWT p=0.0001). However, there were no significant differnences among host choice at 2 hrs (KWT p=0.3507) and 4 hrs (KWT p=0.5161) (Fig. 4.4). Although there were changes throughout the experiment in the most preferred grasses, certain grasses remained favourable throughout 72 hours (e.g. timothy).

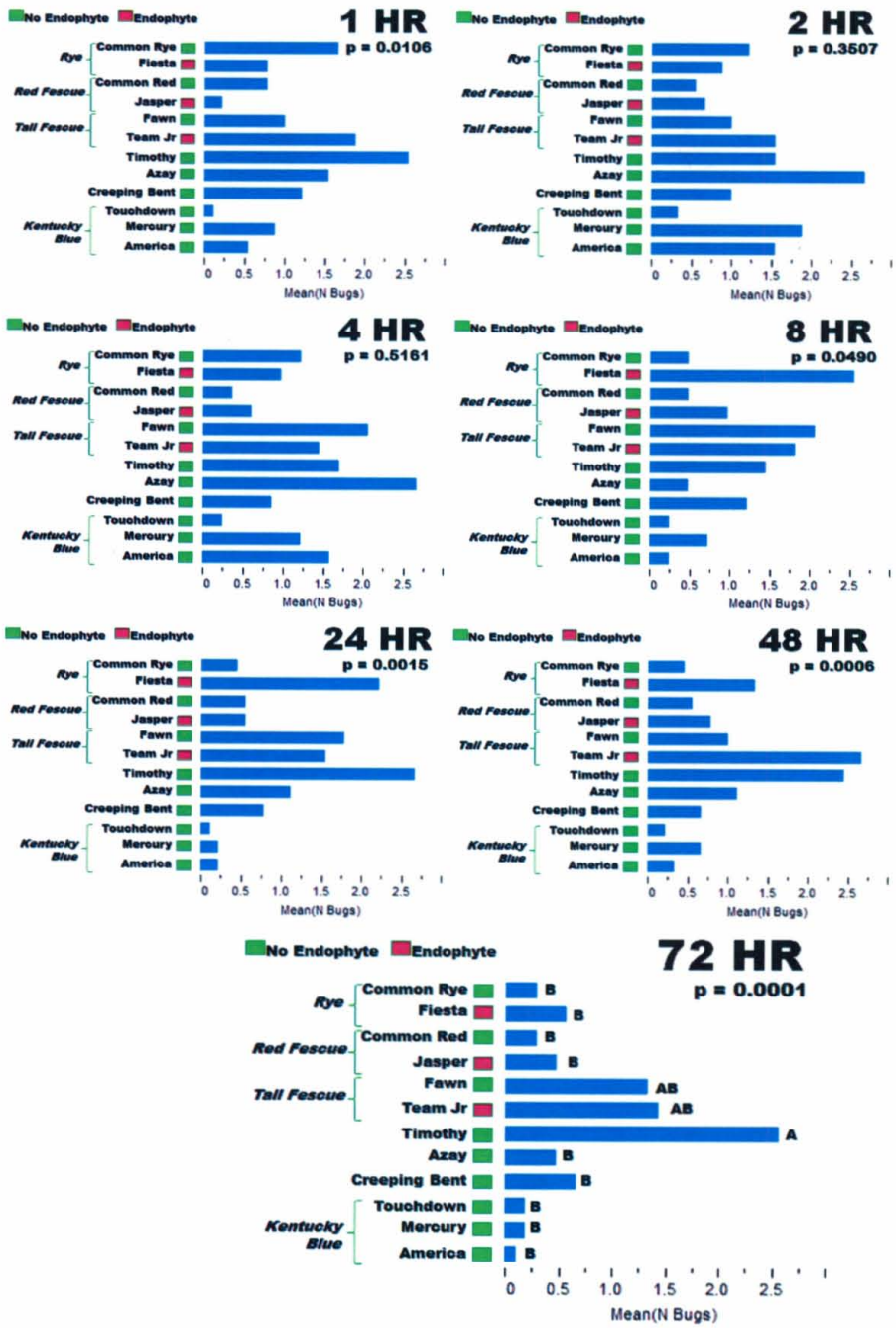

FIGURE 4.4. Mean number of *B. leucopterus hirtus* 4th and 5th instars on 12 different cultivars at t=1, 2, 4, 8, 24, 48 and 72 hours. Additional analysis on the results at 72 hours included a pairwise comparison between tillers. Tillers sharing the same alphabetical notation for results at this time are not significantly different. There were significant differences among the tillers selected at all time intervals except t=2 and 4 hours.

4.3.2 Experiment 2: Comparison of Host Preference in Locally Separated Populations:

In summary, 95 of 200 1st instars made a visible choice at 72 hours. Other individuals were found unassociated with a tiller group or dead. The odds of a single bug choosing a particular cultivar were calculated using a BLR odds ratio test and converted to proportion to compare local populations in St. John's, NL. The proportion of 1st instar nymphs that selected Kentucky bluegrass and sheep fescue varied significantly between site origin (p=0.027 and p=0.048 respectively) (Table 4.4).

TABLE 4.4. Proportion of 1st instar *B. leucopterus hirtus* choosing one of four grass cultivars at four locally separated sites in St. John's, NL. The total number of bugs making a choice at each site were NL1: n=27/50, NL2: n=32/50, NL3: n=21/50, NL4: n =15/50.

St. John's Location	**Initial n**	**Made a choice**			
		Timothy	Kentucky Blugrass	Tall Fescue	Sheep Fescue
NL1	50	12	2	1	12
NL2	50	11	15	1	5
NL3	50	4	6	3	8
NL4	50	1	6	0	8
Differences among sites based on BLR odds ratios		p=0.078	p=0.027	p=0.444	p=0.048

4.3.3 Experiment 3: Comparisons of Host Preference in Regionally Separated Populations:

In this experiment, 124 of 200 1st instars made a choice. Among the cultivars, more than half the insects selected timothy or Kentucky bluegrass (Table 4.5). In a whole model BLR, the proportion of 1st instar nymphs that selected timothy varied significantly between sites (p=0.003). Pair-wise comparison revealed that 1st instar nymphs from the

site in Nova Scotia site selected timothy significantly less frequently than individuals from the other populations.

TABLE 4.5 Proportion of 1st instar *B. leucopterus hirtus* choosing one of four grass cultivars at regoinlly separated sites in Quebec and Atlantic Canada. The total number of bugs making a choice at each site were NL1: n=26/50, NL2: n=29/50, NL3: n=37/50, NL4: n =32/50.

Province	**Initial n**	**Made a choice**			
		Timothy	Kentucky Blugrass	Tall Fescue	Sheep Fescue
PQ	50	15	8	2	1
NB	50	13	8	5	3
NS	50	4	19	8	6
NL	50	11	15	1	5
Differences among instar based on BLR odds ratios converted to proportions		p=0.003	p=0.153	p=0.159	p=0.498

4.3.4 Experiment 4: Comparison of Host Preference between 1st and 5th Instar Nymphs:

The results from this experiment are based on test individuals reared from a single population of *B. leucopterus hirtus* in the Annapolis Valley in Nova Scotia. There was no significant difference in host choice between the initial trials conducted with 5th instars in Experiment 4 (in which there was high mortality) and the second series of trials in Experiment 4, as described above, so these results were combined. The most common choice for 1st instar nymphs was Kentucky bluegrass and a significantly higher proportion of the 1st instar populations chose this grass compared to 5th instars (X^2=9.501, (3), p=0.0233). In contrast, there was no difference in host choice for 5th instars (Table 4.6).

TABLE 4.6. Proportion of 1st instar *B. leucopterus hirtus* and 5th instar *B. leucopterus hirtus* choosing one of four types of grass. Nymphs were reared from adults collected at a single site in the Annapolis Valley, NS. The total number of bugs making a choice for each age group was 1st instar: n=37/50, 5th instar: n=50/104.

Istar	**Initial n**	**Made a choice**			
		Timothy	Kentucky Blugrass	Tall Fescue	Sheep Fescue
1	50	4	19	8	6
5	50	10	10	16	14
Differences among instar based on BLR odds ratios converted to proportions		p=0.150	p=0.015	p=0.228	p=0.150

4.3.5Experiment 5: Comparison of Host Preference between One Individual and Groups of 10 Individuals:

In some cases (New Brunswick and Newfoundland), this resulted in low sample numbers for comparison because of mortality during the study. As a result, only sites from Quebec and Nova Scotia are presented here. In Quebec, only insects making a choice individually selected timothy, and this resulted in a significant difference with those in a group, as the group never selected timothy. In Nova Scotia significantly more grouped insects chose tall fescue (Table 4.7)

TABLE 4.7. Proportion of 5th instar *B. leucopterus hirtus* in PQ and NS choosing one of four types of grass when allowed a choice individually or within a group of 10.

		Initial n	Timothy	Kentucky Bluegrass	Tall Fescue	Sheep Fescue
PQ	Individual	42	13	11	7	11
	Group	50	0	6	4	1
Differences among instar based on BLR odds ratios converted to proportions			p=0.008	p=0.081	p=0.172	p=0.193
NS	Individual	31	8	6	6	11
	Group	50	2	4	10	3
Differences among instar based on BLR odds ratios converted to proportions			p=0.174	p=0.885	p=0.015	p=0.122

4.4 Discussion

4.4.1 Flexibility of Host Choice in *B. leucopterus hirtus*

Blissus leucopterus hirtus exhibited a wide range of host preference in this series of experiments, which is consistent with general observations *of B. leucopterus hirtus* (Ahmad et al. 1984, Ramoska and Todd 1985). In general, host choice varied among both local and regionally separated populations, suggesting host flexibility in *B. leucopterus hirtus.* The origin of these differences may lie in either genetic selection or phenotypic plasticity (itself a potential result of genetic selection). Exposure and learned behavior, which have previously been explored as potential sources of variation in host choice behaviour (Schoonhoven et al. 2005), are not probable causes for observed differences since nymphs were raised in identical conditions. However, it is possible for plastic responses to be maintained across generations (Agrawal et al. 1999), so plasticity remains a possible explanation for observed differences. Regardless of the underlying response mechanism, it is evident that *B. leucopterus hirtus* is capable of adaptation in response to

heterogeneous and/or novel environments; a characteristic that is advantageous for organisms that seek new habitats (Fordyce 2006).

Experiment 1 The experiment was used to identify a range of grasses and cultivars to be used in a more limited comparison of host choice. Because of the combination of cultivars used in this experiment (multiple cultivars from the same species, some endophyte enhanced) it is difficult to make a direct comparison of preference among species. In reality, less data was obtained than originally anticipated. However, the time lapse experiment revealed that timothy was favoured throughout the study whereas other cultivars varied in the degree to which they attracted the bugs over time. All three cultivars of Kentucky bluegrass were widely favoured until t= 4 hours, after which time they quickly diminished in attractiveness. At t=72 hours, the Kentucky bluegrasses tiller appeared stressed (brownish, non-turgid). A similar pattern of stress was seen in common rye and Azay (sheep fescue). This may have been caused by either intense feeding or suboptimal growth conditions. The decrease in apparent health and attractiveness of the endophyte-negative ryegrass appeared to correspond to an increase in attractiveness over time in the endophyte positive rye grass cultivar (Fiesta). It is therefore possible that although the endophyte-negative cultivar of rye was initially preferred, the endophyte-positive cultivar increased in relative attractiveness once the endophyte-negative cultivar became stressed, despite the potential health risk to the insects. This type of preference ranking, documented in several insect species (Carrière and Roitberg 1996, Prokopy et al. 1988), is a process driven by insect sensory perception of potential plant hosts, and is elaborated upon by Papaj and Rausher (1983) and Carrière (1998). It is likely that *B.*

leucopterus hirtus was able to detect differences among plant choices in this experiment, as in previous experiments in which it was able detect endophyte presence at the microscale (Mathias et al. 1990, Carrière et al. 1998). Additionally, a microscale feeding study by Anderson et al (2006) using *B. leucopterus hirtus* examining labial probing (reflecting feeding interest) on multiple grasses including sorghum, fine fescues and buffalograss found more frequent sampling of potential hosts than in other species within the *Blissus* complex.

The cultivars used in *Experiment 1* were structurally distinct. However, the relative importance of structural variation and variation in nutritional quality on host choice is not clear because of evidence of stressed tillers at 72 hours, a time that was selected in order to mirror previous experiments (Eickhoff et al. 2006). This may have affected host choice in the latter hours of this experiment. To account for this, the number of tillers was increased from one to five in subsequent experiments, which resulted in seemingly less stress on the grass over 72 hours. However, further work is needed to determine the instar specific feeding intensity of *B. leucopterus hirtus*, and the rate at which it can cause deterioration of overall plant health. Additional experiments would also help determine if changes in preference by *B. leucopterus hirtus* are related to grass architecture, decreasing nutritional value or changing chemical signals from these grasses. The results of this experiment, however, do highlight the importance of choosing an appropriate time scale for host choice studies, particularly in cases where the food availability is limited within the experiment.

Experiment 2 revealed that locally separated populations in St. John's were significantly different in the probability of *B. leucopterus hirtus* selecting both Kentucky bluegrass and sheep fescue as host plants. The preference for tall fescue was low in all populations, while at other sites, insects appear to select between either sheep fescue or timothy in most cases. This is surprising considering that both cultivars are bunch grasses. However, timothy has coarse and broad leaves while sheep fescue has fine and narrow blades, which could result in differential selection based on tissue penetrability and protective coverage provided. Eickhoff et al. (2006) examined the importance of architecture in the host choice of *Blissus occiduus*. In their twelve-cultivar experiment they found that there was a preference by 4th and 5th instar *B. occiduus* for the stoloniferous grasses such as St. Augustinegrass and bermudagrass that provided protective coverage. Eickhoff et al. (2006) proposed that plant structure could play important roles in insect-plant interactions, beyond feeding.

In some cases, when not feeding, insects may optimize body temperature or shelter by changing environments throughout the day. Such behaviour is exhibited, for example, in the black desert grasshopper, *Taeniopoda eques* Burmeister (Orthoptera: Romaleidae), which roosts on elevated plant parts during sunny periods (Whitman 1987) and descends further down the plant during cloudy days. Lynch et al. (1987) also suggested the variable leaf width of bermudagrass may provide an attractive host for *B. leucopterus leucopterus* because of versatility of the grass as both a feeding source and as shelter, again highlighting the potential importance of plant architecture in host selection. Apart from nutritional considerations, which were not evaluated in this study, it is

possible that extremely delicate 1st instars prefer the thin, pliable blade texture of Kentucky bluegrass for feeding. However, when not engaged in feeding activity, they may prefer the shelter provided by coarser bunch grasses. A more focused study in which leaves were periodically sampled and examined for leaf punctures would yield greater insight into this aspect of *B. leucopterus hirtus* behaviour.

Based on *Experiment 2*, the odds of an individual choosing Kentucky bluegrass were similar in populations from sites NL2, NL3 and NL4, but not from site NL1. The key implication of these results is that, although the reasons for this difference for this are not immediately apparent, the variability in host choice observed in this experiment over these scales emphasizes the importance of future host preference studies from populations that are representative of the local and regional scale, so that host preference within individual populations is not incorrectly extrapolated to larger regions.

Results from regional populations (*Experiment 3*) demonstrated a similar spectrum in host choice compared to *Experiment 2*. There was a significant difference among the sites in the odds of an individual selecting timothy, and although the hierarchy of preference for Kentucky bluegrass, tall fescue and sheep fescue remained relatively consistent, the odds of a bug choosing any of these grasses seemed to be roughly inversely related to the odds of a bug choosing timothy. The exception is at one site in Newfoundland (NL2), where the proportion of individuals selecting tall fescue was very low. From *Experiment 2* however, it appears that avoidance of tall fescue was a common characteristic among populations in St. John's, NL. Because this was a laboratory based experiment, with all grasses grown under identical conditions, it appears that avoidance

of tall fescue is a regional phenomenon. However, an understanding of the reasons for regional variation in host preference will require more detailed study.

In combination, *Experiments 2* and *3* demonstrate that *B. leucopterus hirtus* feeds on a variety of grass hosts and that its preferred host may differ slightly across geographic region. The difference in host choice observed among identically reared populations from different origins, exposed to identically cultured host plants, suggests heritability of host choice, as was found by work on the southern chinch bug *Blissus insularis* in which host adaptation was shown to have genetic influences (Busey 1990). In accordance with *Experiment 2*, it is clear that current methodologies in turf science for determining host choice in *B. leucopterus hirtus* are insufficient for extrapolation to larger scales due to variability among local populations. Experiments of this nature should include multiple replicates to account for regional modes and variance, and population samples should be taken from a diversity of habitats across the insect's range. A parallel study that incorporates aspects of geographic isolation should also be conducted in Newfoundland, as this island is separated from the North American mainland, with prevailing climatic differences.

Variability in host choice and in life history are key underpinnings in the 'here and now', 'here and later' or 'elsewhere' templet model (Southwood 1977, Solbreck 1978), which predicts that insect species will adapt in a variety ways to a dynamic and heterogeneous environment. The genetic and/or phenotypic flexibility of characteristics is likely one of the characteristics that has made *B. leucopterus hirtus* so effective as a wide ranging pest species. The capacity for plasticity in host choice is likely an evolutionary

response to the dynamics of the environmental conditions and that of the potential hosts across the range of *B. leucopterus hirtus*. Being aware of this potential flexibility and its possible determinants should assist pest managers by providing awareness of multiple potential hosts that may differ with geographic locality and changing climate conditions. For example, pest researchers may better predict where (in which turf areas) to search for aggregated populations of *B. leucopterus hirtus* as they emerge from sheltered location in the spring, thrive in feeding grasses during early summer, and aggregate to more protective grasses in the fall.

4.4.2 Changes in Host Preference with Ontogeny of *B. leucopterus hirtus.*

The results of *Experiment 4* clearly showed that host choice changed significantly between 1st and 5th instar nymphs. First instar nymphs had a preference for Kentucky bluegrass whereas 5th instars were found more evenly distributed among hosts. Phonologically based intra-population variation in host choice is also seen in other insect groups. As outlined in the chapter introduction, research on other insect orders provides evidence that insects may feed on different plants during different periods of their ontogeny, reflecting differences in nutritional, protective or other biotic/abiotic requirements. It appears, based on these results, that age affects host choice and, in the case of *B. leucopterus hirtus*, young instars are partial to Kentucky bluegrass. This is likely due to nutritional, textural and/or architectural characteristics of this grass. The soft, fine texture of Kentucky bluegrass is likely appealing to the young, fragile nymphs of *B. leucopterus hirtus* that possess very small mouth parts. The delicate nature of the

young insects was noted by the researchers as they attempted to move and manipulate the young insects.

As the insects age and the season approaches autumn, a move away from soft, succulent grass such as Kentucky bluegrass may represent an optimized overwintering strategy in response to a seasonal climate (i.e move to more protective areas). Adults of *B. leucopterus hirtus* are known to overwinter under debris and within the dense structure of bunch grasses (Vittum et al. 1999). As the insects mature they become increasingly aggregated (Mailloux and Streu 1982). It is, therefore, possible that a shift by 5th instars away from Kentucky bluegrass may, in fact, represent a movement towards more protective grasses. However, this does not fully explain why aggregation occurs in populations that experience two cohorts per year (Mailloux and Streu 1982). If it is true that shifts away from preferred feeding hosts occur, reduction in nutritional quality or increase in feeding effort that may result from a shift in host species may be offset by fitness of protective space (Diamond and Kingsolver 2010). The random distribution among the several grasses available suggests that choice may be strongly influenced by aggregation drivers (e.g. pheromones – not yet identified in *B. leucopterus hirtus*) over grass qualities. Although the biological explanation for age-related host preference shifts is not yet clear for *B. leucopterus hirtus*, it is apparent that age of instar must be taken into account in all future studies of host preference in *B. leucopterus hirtus*. For example, the results of Eickhoff et al. (2006), in which 4th and 5th instars were used, must be taken in context, especially since it appears choice in young instars is more selective. Unfortunately, testing using 2nd, 3rd and 4th instar nymphs and adults was not possible in

this experiment due to a lack of specimens. Further investigation into age-specific shifts in host choice should involve representation by all instars and consideration of plant physiology as well.

4.4.3 Group Dynamics in Host Choice Behaviour of *B. leucopterus hirtus*

Data from the final experiment (*Experiment 5*), in which 5th instars were allowed to choose a host either alone or in a group of 10, were challenging to interpret. Host choice in both these scenarios was significantly different among regions and it was, therefore, necessary to examine each region separately. This resulted in small n values for each set of regional data, and based on this, the decision was made to include only populations from Quebec and Nova Scotia in the analysis, since they had the highest number of data points. Additional replication among all groups would have strengthened the investigation. The statistical analysis revealed no significant differences in host preference between an isolated individual and individuals within groups. Taken at face value, this suggests that individuals of this age, regardless of density, may seek out similar hosts. However, choice among individual insects did appear to be more evenly distributed among the grass types than it did for insects choosing as a group. This is somewhat different from the results in *Experiment 4*, where the choice of grouped instars appears fairly randomly distributed. Further investigation is required to elucidate relationships between individual and group choice behaviour.

Experiment 5 did suggest that multiple hosts may be suitable for *B. leucopterus hirtus* throughout its life history. The results of this experiment suggest that *B.*

leucopterus hirtus has a wide range of potential hosts and may base selection on multiple criteria, including nutritional quality and shelter provision. This flexibility may be an adaptive behaviour intended to counter the negative effects of high density feeding and subsequent resource quality on individual insect fitness (Jaenike 1990). However, it is also possible that the number of bugs used in this experiment to reflect a higher density (10 within an arena) was simply not adequate to evoke the physiological or behavioural effects of higher densities in *B. leucopterus hirtus*.

The methods employed in *Experiment 1-5* were modelled after previous work by Eickhoff et al. (2006). There are several aspects for consideration to improve future host choice studies of this nature. For example, handling of 1st instar nymphs required extreme delicacy and perhaps caused an increase in early mortality which could have been resolved by transferring eggs into experimental arenas before hatch. The high mortality of 5th instars remains unexplained, although it is possible that the insects were either too old, too stressed or underfed at the commencement of the experiment. In this experiment, the definition of 'having made a choice' was defined by finding an insect within 1 cm of a plant in an attempt to account for insects which fell off the tillers in the process of moving the colonies. The 1 cm buffer was an 'inclusive' measure of choice and results may have been different if choice had been measured more strictly (e.g. must be found on the plant). Although it appeared that deteriorating quality of the plants over 72 hours may have been mitigated by increasing the number of tillers available for feeding, further experiments may determine the optimum quantity of food for timed host choice trials. It would be interesting to investigate the instar specific feeding intensity of *B. leucopterus*

hirtus to know whether the condition of the grass at 72 hours was directly attributable to insect feeling, or may have be caused by environmental conditions including lack of nutrients. As a final note, it is important to clarify, as has been done previously (Eickhoff et al. 2004), that all of the above experiments involved testing only one cultivar among many within several species of grass. These results only demonstrate experimentally the variability in host preference among various populations in the laboratory and require additional research in the laboratory and field before they can be applied to pest management. However, with the current knowledge gained through this experiments, pest managers should have a better understanding of how choice and behaviour may differ with age and geographical region. This could be extremely beneficial to managers attempting techniques such as vacuum suction to collect populations of *B. leucopterus hirtus* when they are most aggregated. (i.e. preferred feeding choice may not be the preferred site for aggregation).

4.5 Summary

This series of experiments was intended as an investigation to lay the ground work for host preference research in *B. leucopterus hirtus*. It is an important component of life history strategy, affected by extrinsic factors at the local and regional scale and/or intrinsic factors such as age and group size. As predicted population variability in host preference was found at both the local and regional level. However, there have been no conclusions drawn on the factors driving these difference. Also predicted, there were difference in the host choice among instars of different ages within the same population, likely reflecting difference need in both nutrition and shelter. It was clear that both

population of origin and age and instar should be taken into account when designing trials and interpreting results related to host selection. The results of individual versus group host choice behaviour were less clear, although the initial results obtained suggested cultivar selected was less important than the benefits of aggregation. These findings should influence future study designs to increase both range of sampling and replication of experiments. Further study will also be needed to determine what specific environmental factors are influencing host choice at the local and regional level as well as genetic variability among populations at local and regional scales which may also influence host preference at various spatial scales.

Literature Cited

Abrahamson, W. G., M. D. Eubanks, C. P. Blair, and A. V. Whipple. 2001. Gall flies, inquilines and goldenrods: a model for host race formation and sympatric speciation. American Zoologist 41:928-938.

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defenses in animals and plants. Nature 401:60-63.

Ahmad, R.., S. Kindler, and K. Pruess. 1984. Recovery of two sorghum varieties from sublethal infestations of chinch bug, *Blissus leucopterus leucopterus* (Say) (Hemiptera: Lygaeidae). Journal of Economic Entomology 77:151-152.

Anderson, W. G., T. M. Heng-Moss, F. P. Baxendale, L. M. Baird, G. Sarath, and L. Higley. 2006. Chinch bug (Hemiptera: Blissidae) mouthpart morphology, probing frequencies, and locations on resistant and susceptible germplasm. Journal of Economic Entomology 99:212-221.

Awmack, C. S., and S. R. Leather. 2002. Host plant quality and fecundity in herbivorous insects. Annual Review of Entomology 47:817-844.

Baker, G. T., X. Chen, and W. K. M. Peter. 2008. Labial tip sensilla of *Blissus leucopterus leucopterus* (Hemiptera: Blissidae): Ultrastructure and behavior. Insect Science 15:271-275.

Baker, P. B., R. H. Ratcliffe and A. L. Steinhauer. 1981. Tolerance to hairy chinch bug feeding in Kentucky bluegrass. Environmental Entomology 10:153-157.

Berlocher, S. H., and J. L. Feder. 2002. Sympatric speciation in phytophagous insects: moving beyond controversy? Annual Review of Entomology 47:773-815.

Bernays, E. A., and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman & Hall, New York, New York, USA.

Bernays, E., and M. Graham. 1998. On the evolution of host specificity in phytophagous arthropods. Ecology 69:886-892.

Bourassa, S., J. Brodeur, and Y. Carrière. 2007. Endophyte-grass complexes and the relationship between feeding preference and performance in a grass herbivore. Entomologia Experimentalis et Applicata 124:221-228.

Braendle, C., G. K. Davis, J. A. Brisson, and D. L. Stern. 2006. Wing dimorphism in aphids. Heredity 97:192-199.

Breen, J. P. 1994. *Acremonium* endophyte interaction with enhanced plant resistance to insects. Annual Review of Entomology 39:401-423.

Brown, W.J. 1949. Collected Specimen. 1 female, St. John's, 16.VII. Canadian National Collection, Ottawa, Ontario, Canada.

Busey, P. 1990. Inheritance of host adaptation in the southern chinch bug (Hemiptera: Lygaeidae). Annals of the Entomological Society of America 83:563-567.

Busey, P., and B. J. Center. 1987. Southern chinch bug (Hemiptera: Heteroptera: Lygaeidae) overcomes resistance in St. Augustinegrass. Journal of Economic Entomology 80:608-611.

Caillaud, M. C., and S. Via. 2000. Specialized feeding behaviour influences both ecological specialization and assertive mating in sympatric host races of pea aphids. American Naturalist 156:606-621.

Carrière, Y. 1998. Constraints on the evolution of host choice by phytophagous insects. Oikos 82:401-406.

Carrière, Y., A. Bouchard, S. Bourassa, and J. Brodeur. 1998. Effect of endophyte incidence in perennial ryegrass on distribution, host choice, and the performance of the hairy chinch bug (Hemiptera: Lygaeidae). Journal of Economic Entomology 91:324-328.

Carrière, Y., and B. D. Roitberg. 1995. Evolution of host-selection behaviour in insect herbivores: genetic variation and covariation in host acceptance within and between two populations of *Choristoneura rosaceana* (Family: Tortricidae), the obliquebanded leadfoller. Heredity 74:357-368.

Carrière, Y., and B. D. Roitberg. 1996. Optimality modelling and quantitative genetics as alternatives to study the evolution of foraging behaviours in insect herbivores. Evolutionary Ecology 10:289-305.

Clay, K. 1996. Interactions among fungal endopyhtes, grasses and herbivores. Researches on Population Ecology 38:191-201.

Cocroft, R. B., R. L. Rodriguez, and R. E. Hunt. 2010. Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. Biological Journal of the Linnean Society 99:60-72.

Denno, R., and B. Benrey. 1997. Aggregation facilitates larval growth in the neotropical nymphalid butterfly *Chlosyne janais*. Ecological Entomology 22:133-141.

Diamond, S. E., and J. G. Kingsolver. 2010. Fitness consequences of host plant choice: a field experiment. Oikos 119:542-550.

Dicke, M. 1994. Local and systemic production of volatile herbivore-induced terpenoids: Their role in plant-carnivore mutualism. Journal of Plant Physiology 143:465-472.

Egan, S. P., P. Nosil, and D. J. Funk. 2008. Selection and genomic differentiation during ecological speciation-isolating the contributions of host-association via a comparative genome scan of *Neochlamisis bebbianae* leaf beetles. Evolution 62:1162-1181.

Eickhoff, T. E. 2002. Turfgrass, crop and weed hosts of the chinch bug, *Blissus occiduus*. M.S. Thesis. University of Nebraska-Lincoln, Lincoln, Nebraska, USA.

Eickhoff, T. E., F. P. Baxendale, and T. M. Heng-Moss. 2006. Host preference of the chinch bug, *Blissus occiduus*. Journal of Insect Science 6:1-6.

Eickhoff, T. E., F. P. Baxendale, T. M. Heng-Moss, and E. E. Blankenship. 2004. Turfgrass, crop, and weed hosts of *Blissus occiduus* (Hemiptera: Lygaeidae). Journal of Economic Entomology 97:63-73.

Fordyce, J. A. 2006. The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. The Journal of Experimental Biology 209:2377-2383.

Funk, D. J., and E. A. Bernays. 2001. Geographic variation in host specificity reveals host range evolution in *Uroleucon ambrosiae* aphids. Ecology 82:726-739.

Futuyma, D. J., and S. C. Peterson. 1985. Genetic variation in the use of resources by insects. Annual Review of Entomology 30:217-238.

Grace, T., S. M. Wisely, S. J. Brown, F. E. Dowell, and A. Joern. 2010. Divergent host plant adaptation drives the evolution of sexual isolation in the grasshopper *Hesperotettix viridis* (Orthoptera: Acrididae) in the absence of reinforcement. Biological Journal of the Linnean Society 100:866-878.

Görür, G. 2005. The importance of phenotypic plasticity in herbivorous insect speciation. Pages 145-171 *in* D. Whitman, and T. N. Ananthakrishnan, editors. Insect Phenotypic Plasticity, Diversity in Responses. Science Publishers Inc. Enfield, New Hampshire, USA.

Görür, G., C. Lomonaco, and A. Mackenzie. 2007. Phenotypic plasticity in host choice behaviour in black bean aphid, *Aphis fabae* (Hemiptera: Aphididae). Arthropod-Plant Interactions 1:187-194.

Gotthard, K., N. Margraf, and M. Rahier. 2003. Geographic variation in oviposition choice of a leaf beetle: the relationship between host plant ranking, specificity, and motivation. Entomologia Experimentalis et Applicata 110:217-224.

Grossmueller, D. W., and R.C. Lederhouse. 1985. Oviposition Site Selection: An aid to rapid growth and development in the tiger swallowtail butterfly, *Papilo glaucus*. Oecologia 66:68-73.

Gullan, P. J., and P. S. Cranston. 2005. The Insects: An Outline of Entomology, 3rd ed. Blackwell Publishing Ltd, Malden, Massachusetts, USA.

Headlee, T. J., and J. Walker. 1913. The chinch bug (*Blissus leucopterus* Say). Kansas State Agricultural College Agriculture Experiment Station Bulletin 91:287-353.

Inouye, B. D., and D. M. Johnson. 2005. Larval aggregation affects feeding rate in *Chlosyne poecile* (Lepidoptera: Nymphalidae). Florida Entomologist 88:247-252.

Jaenike, J. 1990. Host specialization in phytophagous insects. Annual Review of Ecology and Systematics 21:243-275.

Karban, R., and I. T. Baldwin. 1997. Induced responses to herbivory. University of Chicago Press, Chicago, Illinois, USA.

Lamp, W. O., and T. O. Holtzer. 1980. Distribution of overwintering chinch bugs *Blissus leucopterus leucopterus* (Hemiptera: Lygaeidae). Journal of the Kansas Entomological Society 53:320-324.

Leonard, D. E. 1966. Biosystematics of the *leucopterus* complex of the genus *Blissus* (Hemiptera: Lygaeidae). Bulletin - Connecticut Agricultural Experiment Station 677:1-47.

Lynch, R.E., S. Some, I. Dicko, H. D. Wells, and W. G. Monson. 1987. Chinch bug damage to bermudagrass. Journal of Entomological Science 22:153-158.

Mailloux, G., and H. T. Streu. 1982. Spatial pattern of hairy chinch bug (*B. leucopterus hirtus* Montandon: Hemiptera: Lygaeidae) populations in turfgrass. Annals of the Entomological Society of Quebec 27:111-131.

Mathias, J. K., R. H. Ratcliff and J. L. Hellman. 1990. Association of endophytic fungus in perennial rye grass and resistance to the hairy chinch bug (Hemiptera: Lygaeidae). Journal of Economic Entomology 83:1640-1646.

Mize, T. W., and G. E. Wilde. 1986a. New resistance germplasm to the chinch bug (Heteroptera: Lygaeidae) in grain sorghum: contribution of tolerance and antixenosis as resistance mechanisms. Journal of Economic Entomology 79:42–45.

Mize, T. W., and G. E. Wilde. 1986b. New grain sorghum sources of antibiosis to the chinch bug (Heteroptera: Lygaeidae). Journal of Economic Entomology 79:176–180.

Nosil, P., C. P. Sandoval, and B. J. Crespi. 2005. The evolution of host preference in allopatric vs. parapatric populations of *Timema cristinae* walking sticks. Journal of Evolutionary Biology 19:929-942.

Nylin, S., and K. Gotthard, 1998. Plasticity in life history traits. Annual Review of Entomology 43:63-83.

Packard, C. M. 1937. How to fight the chinch bug. Farmer's Bulletin: U.S. Department of Agriculture 1780:1-21.

Papaj, D. R. 1986. Interpopulation differences in host preference and the evolution of learning in the butterfly, *Batthus philenor*. Evolution 40:518-530.

Papaj, D. R., and M. D. Rausher. 1983. Individual variation in host location by phytophagous insects. Pages 77-124 *in* S. Ahmad, editor. Herbivorous insects: host seeking behaviour and mechanisms. Academic Press, New York, USA.

Pener, M. P., and Y. Yerushalmi. 1998. The physiology of locust phase polymorphism: an update. Journal of Insect Physiology 44:365-377.

Potter, D. A. 1998. Destructive turfgrass insects: biology, diagnosis and control. Ann Arbor Press, Chelsea, Michigan, USA.

Prokopy, R. J., S. P. Diehl, and S. S. Cooley. 1988. Behavioural evidence for host races in *Rhagoletis pomonella* flies. Oecologia 76:138-147.

Ramoska, W. A. and Todd, T. 1985. Variation in efficacy and viability of *Beauveria bassiana* in the chinch bug (Hemiptera: Lygaeidae) as a result of feeding activity on selected host plants. Environmental Entomology 14:146-148.

Richmond, D. S., and D. J. Shetlar. 2000. Hairy chinch bug (Hemiptera: Lygaeidae), damage, population density and movement in relation to the incidence of perennial ryegrass infected by Neotyphodium endophytes. Journal of Economic Entomology 93:1167-1172.

Rothschild, M., and L. M. Schoonhoven. 1977. Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae). Nature 266:352-355.

Saha, D. C., J. M. Johnson –Cicalese, P. M. Halisky, M. I. Van Hemmstra, and C. R. Funk. 1987. Occurrences and significance of endophytic fungi in the fine fescue. Plant Disease 71:1021–1024.

Sarkar, S. K., and H. Midi. 2010. Importance of assessing the model adequacy of binary logistic regression. Journal of Applied Science 10:479:486.

Schoonhoven, L. M., T. Jermy, and J. J. A. van Loon, 1998. Insect and flowers: The beauty of mutualism. Pages 315-342 *in* Insect-Plant Biology: From Physiology to Evolution. Chapman & Hall, London, UK.

Schoonhoven, L., J. J. A. van Loon and M Dicke. 2005. *Insect-Plant Biology*. Oxford University Press, Oxford.

Scriber, J. M. 1994. A new 'cold pocket' hypothesis to explain local host preference shifts in *Papilio canadensis*. Entomologia Experimentalis et Applicata 80:315-319.

Singer, M. C., C. Stefanescu, and I. Pen. 2002. When random sampling does not work: standard design falsely indicates maladaptive host preference in a butterfly. Ecology Letters 5:1-6.

Slansky, F. Jr., and J. M. Scriber. 1985. Food consumption and utilization. Pages 87-163 *in* G. A. Kerkut, and L. I. Gilbert, editors. Comprehensive insect physiology, biochemistry and pharmacology vol. 4. Pergamon, NewYork, New York, USA.

Solbreck, C. 1978. Migration, diapause, and direct development as alternative life strategies in a seed bug, *Neacoryphus bicrucis*. Pages 195-217 *in* H. Dingle, editor. Evolution of insect migration and diapause. Springer-Verlag, New York, New York, USA.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology 46:336-365.

Stockoff, B. A. 1993. Ontogenetic change in dietary selection for protein and lipid by gypsy moth larvae. Journal of Insect Physiology 39:677-686.

Strauss, S. Y., and R. Karban. 1998. The strength of selection: intraspecific variation in host-plant quality and the fitness of herbivores. Pages 156–180 *in* S. Mopper and S. Y. Strauss, editors. Genetic structure and local adaptation in natural insect populations: effects of ecology, life history and behavior. Chapman and Hall, New York, New York, USA.

Vanbergen, A. J., B. Raymond, I. S. K. Pearce, A. D. Watt, R. S. Halls, and S. E. Hartley. 2003. Host shifting by *Operophtera brumata* into novel environments leads to population differentiation in life history traits. Ecological Entomology 28:604-612.

Vittum, P., M. Villani, and H. Tashiro, 1999. Turfgrass insects of the United States and Canada, 2nd ed. Cornell University Press, New York, USA.

Way, M. J., and M. Cammell. 1970. Aggregation behaviour in relation to food utilization by aphids. Pages 229-247 *in* A. Watson, editor. Animal populations in relation to their food resources. Blackwell, Oxford, UK.

Wehling, W. F., and J. N. Thompson. 1997. Evolutionary conservatism of oviposition preference in a widespread polyphagous insect herbivore, *Papilio zelicaon*. Oecologia 111:209-215.

Wennström, A, L. N. Hjulström, J. Hjältén and R. Julkunen-Tiitto. 2010. Mother really knows best: host choice of adult phytophagous insect females reflects a within-host variation in suitability as larval food. Chemoecology 20:35-42.

Whitman, D. W. 1987. Thermoregulation and daily activity pattern in the black desert grasshopper, *Taeniopoda eques*. Animal Behaviour 35:1814-1826.

Chapter 5: Summary and Concluding Remarks

Blissus leucopterus hirtus Montandon (Hemiptera: Blissidae) (hairy chinch bug) (Fig. 1.1), is a widespread turfgrass pest in Quebec and Atlantic Canada that causes considerable damage to turf and lawns. Many aspects of its life history were not well understood, including difference in life history strategy across its geographic range. This research investigated the influence of environmental heterogeneity on the phenology, morphology and host preference of *B. leucopterus hirtus* at a local and regional level. Southwood's habitat as a templet model, which he proposed in his seminal address to the British Entomological Society in 1977 (Southwood 1977) outlined life history strategies that would maximize the fitness in insects under various selective pressures. In this thesis, Southwood's model was used as a conceptual framework for studying the variability of the response of *B. leucopterus hirtus* to unique environmental conditions, which was consistent with various applications of the habitat templet model (Campbell et al. 1974, Southwood 1977, Solbreck 1978). This approach was taken for the purpose of providing a sounder scientific database from which to plan ecologically based management strategies. Using knowledge gathered from previous, less ecologically focused studies, predictions were made regarding patterns in phenology, sex ratio, wing form, size components and feeding flexibility. It is clear from the results that difference in life history, morphometrics and host choice behaviour varied at the regional, and sometimes local, level. This may be the result of either adaptive selection or phenotypic and behavioural plasticity in this pest. Further experiments will need to elucidate which of

these two dominates, but if phenotypic plasticity is strongly present, capacity to adapt to changing environments could be extremely rapid.

In *Chapter 2, Local and Regional Variation in Phenology of Blissus leucopterus hirtus Montandon (Hemiptera: Blissidae),* it was shown that the rate of egg development differed locally and, to a greater degree regionally, in an apparent response to climate conditions. The lower threshold for development and faster development rate of eggds in St. John's, NL, relative to other sampled location, demonstrates evidence that the insects has compensated for a cooler, shorter summer. Comparison of methods for measuring phenology in this pest provided similar results in predicting peak occurrence of instars. In St. John's, Newfoundland, there was little difference between phenology of *B. leucopterus hirtus* at 10 sites, providing little evidence of variation at the local scale. However, when pooled data from St. John's were compared with other areas across larger geographical regions (NB, NJ) , differences in peak instar occurrence were found, suggesting variation in phenology did exist at the regional scale. This evidence suggests that *B. leucopterus hirtus* is capable of adaptation at a climatic scale, particularly along northern latitudes and is a potential invasive threat at more northern latitudes. However, with greater emphasis on the influence of climate on these ontological patterns, pest managers should be able to employ moe standardized productive techniques as those used in this study and employ the results to apply necessary chemical spray at the appropriate interval (e.g. When *B. leucopterus hirtus* are most susceptible at 2^{nd} and 3^{rd} instars). The research results indicate that as predicted by the habitat templet model, and in accordance with the several hypotheses of this research *B. leucopterus hirtus* showed adaptive

changes or plasticity in its life history across its geographic range in response to differing habitat characteristics, including a lower threshold for development and faster egg development rate compared to *B. leucopterus hirtus* in other parts of the insect's range.

The findings of *Chapter 3: Local and Regional Variation in Sex Ratio, Wing Morph and Mensural Characters of Blissus leucopterus hirtus* add additional perspective to the adaptive response of *B. leucopterus hirtus* under differing local and regional conditions. The sex ratio of *B. leucopterus hirtus* was consistent across collection periods and locations, indicating a stable operational sex ratio of approximately F:M=1:1.19. Thus, sex ratio was not an indicator of habitat differentiation at either the local or regional scale. However, a significantly greater proportion of males in Fall 2004 and Fall 2005 suggest either differential mortality of dispersal over the summer time frame. This combined with the fact that there are significantly more male brachypters than female brachypters suggest that macropterous females may possess a dispersal advantage. Wing form did vary locally separated populations and in the same populations over time, suggesting a response to differences in local and regional level environmental conditions among these habitats. Wing form ratios, therefore, have the potential to inform pest managers about the conditions and permanence of populations, as brachypterous individuals tend to be produced when the habitat has adequate resources for the population. Unfortunately, the results from this study did not show statistical evidence to support a relationship between either growth rate or developmental threshold to average size ranking.

Chapter 4: Host Choice Behaviour of Blissus leucopterus hirtus in Relation to Geographic Distribution, Insect Age and Insect Group Size provided insights into host choice. A series of preliminary experiments evaluated host preference in *B. leucopterus hirtus* in relation to extrinsic factors at the local and regional scale and/or intrinsic factors such as age and group size. Population variability in host preference was found at both the local and regional level, and between instars of different ages within the same population. It was clear that both population of origin and age of instar should be taken into account when designing experiments to determine host choice. As the rank order of preference may change over an insect's life time, it would be helpful to pest managers if this order of preference was taken into account. For example, the results of individual vs. group host choice behaviour were mixed, although the initial data obtained suggested cultivar selected was influenced by the benefits of aggregation. It is likely, based on this research that instars are likely to be found in proximity to Kentucky bluegrass in the early parts of the summer seasn, but found increasingly aggregated in other grasses near fall. This also opens the possibility of employing non chemical measures such as vacuuming to capture large number of aggregated individuals.

In conclusion, the range of climatic conditions across the Canadian distribution of *B. leucopterus hirtus* provided an suitable opportunity to explore the effect of climate variation on the life strategy of this insect. Together, the results of this research provide a strong case to support the adaptability of *B. leucopterus hirtus* to a variety of habitats at both the local scale in St. John's, NL, and at the regional scale across Quebec and Atlantic Canada. There were notable adaptations by B. leucopterus populations in St.

John's including a marked difference in threshold temperature for egg development and rate of egg development compared with other areas in the in the insect's range. This ability to thrive in a range of habitats may be the result of adaptive selection or phenotypic and behavioural plasticity. Regardless of the underlying mechanism driving this flexibility, these results highlight the potential for this species to adapt to new and changing. While there has been little attention given to the complete ecology of *B. leucopterus hirtus* in previous pest management work, this thesis has uncovered some clear patterns among populations that subscribe to Southwood's habitat templet and therefore provide a contextual framework in which to understand the patterns observed,

Based on the finding in this thesis, the following recommendation for researchers and pest managers are made concerning *B. leucopterus hirtus*:

1. Phenological models for *B. leucopterus hirtus* based on data collected in Fredericton, New Jersey and Ontario do no accurately reflect phenology of *B. leucopterus hirtus* in St. John's, NL. Populations in this study area are univoltine and generally more "compact" than life cycles in other regions.

2. Researchers and pest managers should expect that egg development of this insect will occur at a lower temperature and at a more rapid rate in St. John's, NL than in other areas of the insect's range. Therefore, periods of warm weather in St. John's, NL can be expected to produce rapid advancements in phenology of *B. leucopterus hirtus* compared to other regions, and early chemical spray times can be expected. Based on the results in 2004 and 2005,

both Julian day and cumulative degree day should provide similar predictions of emergence for 2nd and 3rd instar, but in situations where especially warm period occur, as mentioned above, cumulative degree days may be an more accurate predictor of appearance.

3. Although sex ratios are fairly consistent across the entire study region (M: F 1:1.19), there were more males found in the fall populations of 2004 and 2005, indicating either differential mortality or dispersal by females. The fact that there are significantly more brachypterous males than females (and therefore more females macropterous) suggests that wing form may relate to dispersal, although this is not confirmed. Based on Southwood (1977) prediction however, wing form may be an accurate predictor of population permanence, with high levels of macropters indicating unstable or heterogeneous populations. In this study it was also found that levels of brachyptery increased over a two year period within the study populations. Therefore pest managers should be aware of the potential for high levels of brachyptery to indicate stable populations, meaning that these populations are liekly to be present for longer periods of time (and potentially over several seasons.)

4. Host choice is very flexible among populations of *B. leucopterus hirtus*, and there is evidence that the insect may employ different varieties or cultivars of grass to accommodate various needs throughout their ontogeny. It is recommended that pest managers search for young instars among the blends

including Kentucky bluegrass that are favourable to feeding. However, at the insects mature, they tend to aggregate and, towards cooler weather in particular, seek shelter, Insects are most more likely to be found during these periods in coarser more dense grasses and even in non-biotic spaces, such as under shingles and yard furniture. This provides another opportunity for non-chemical control such as vacuuming the insects. Another important result from this research is that in preliminary observations, it appears aggregation at the 5th instar can be a more powerful that host preference alone. Again,. This could have important implications for pest managers if alternative methods for attracting B. leucopterus to a single point can be achieved.

This thesis has taken a broad approach to gather ecological intelligence regarding an insect pest species that although fairly common, is poorly understood. By exploring aspects of the insects phenology, morphology and host choice at a local and regional scale, differences in these measure have been identified. In some cases, these results have been directly comparable to those existing from previous research, and larger conclusions have been drawn regarding patterns in ontology and life history. Where previous research was lacking, results from this study have been compared with results in the literature with potential explanations for similar of different results proposed. In many cases, particularly in the case of host choice, results regarding the difference in host choice by age and/or group size emphasize the importance of these factors taken into account during turf-resistance trials, which are the focus of large agricultural research initiatives. With the addition of this research as a whole, a clearer picture has now emerged of how

environment influences the life history, morphology and host choice of *B. leucopterus hirtus*.

Literature Cited

Campbell, A., B. D. Frazer, N. Gilbert, A. P. Gutierrez, and M. Mackauer. 1974. Temperature requirements of some aphids and their parasites. Journal of Applied Ecology 11:431-438.

Solbreck, C. 1978. Migration, diapause, and direct development as alternative life strategies in a seed bug, *Neacoryphus bicrucis*. Pages 195-217 *in* H. Dingle, editor. Evolution of insect migration and diapause. Springer-Verlag, New York, New York, USA.

Southwood, T. R. E. 1977. Habitat, the templet for ecological strategies? Journal of Animal Ecology 46:336-365.

Appendix A. Latitude and Longitude of Sampling Locations in St. John's, NL and across Quebec and Atlantic Canada

TABLE A1. Geographical coordinates for local sampling sites in St. John's collected at time of original sampling using a handheld GPS unit. Coordinates are based on WGS84 datum.

Local Site	Latitude	Longitude
A	47.587168	-52.703686
B	47.593433	-52.719203
C1	47.56811	-52.72874
C2	47.59274	-52.724694
D	47.568208	-52.73007
E	47.553625	-52.735613
F	47.5536	-52.736694
G	47.532884	-52.771835
H	47.529816	-52.778184
I	47.513809	-52.780136
J	47.514707	-52.784385

TABLE A2. Geographical coordinates for regional sampling sites across Quebec and Atlantic Canada collected at time of original sampling using a handheld GPS unit. Coordinates are based on WGS84 datum.

Regional Site	Latitude	Longitude
PQ1	46.845653	-71.359942
PQ2	46.771135	-71.316227
PQ3	46.940987	-71.312744
NB1	45.924384	-66.609297
NB2	45.916233	-66.596611
NB3	45.383396	-65.979157
NS1	45.067078	-64.498313
NS2	45.084762	-64.478982
NS3	45.08236	-64.36295
NL1	47.5293	-52.778538
NL2	47.570511	-52.725475
NL3	47.594233	-52.722702

Appendix B. Distribution of *Blissus leucopterus hirtus* Montandon (Hemiptera: Blissidae) on the Island of Newfoundland

Introduction

Blissus leucopterus hirtus Montandon (Hemiptera: Blissidae) (hairy chinch bug) is a serious turfgrass and crop pest in Quebec and Atlantic Canada, and the northeastern United States (Leonard 1966, Vittum *et al.* 1999, Potter 1998). In Canada, *B. leucopterus hirtus* is a widely distributed destructive turf pest in Ontario, Quebec and the Maritime Provinces, which includes New Brunswick, Nova Scotia and Prince Edward Island. In the last decade it has also become a serious turfgrass pest in St. John's, Newfoundland, the capital city of an island off the East coast of Canada. The species was first recorded in Newfoundland in 1949 (Brown 1949), the year the province joined the Canadian Confederation. Whether its presence on the island is the result of an introduction from the mainland of Canada, or due to better faunal surveys conducted by the Government of Canada is not certain. The range of this species on the island of Newfoundland is unknown, and this study aims to determine the presence/absence *of B. leucopterus hirtus* in several communities across Newfoundland.

Materials and Methods

Over the course of one week, in mid-September 2005, ten sites across the island were sampled using a BioQuip® handheld vacuum sampler. The collection cup of the vacuum

was modified by temporarily inserting a section of nylon pantyhose tied at one end, which improved the capture and handling of these insects. Areas within the selected urban locations exhibiting classic *B. leucopterus hirtus* damage (large brown patches) were swept with the vacuum sampler for approximately thirty seconds, with focus on the outer edges of the patches, where the insects were most often aggregated. Debris was emptied from the pantyhose into a plastic tray, the debris sorted, and any *B. leucopterus hirtus* found placed in sealed vials with 70% ethanol labeled with the date, location and time. In sample sites where *B. leucopterus hirtus* populations were low or absent, up to four additional sites within the urban area were sampled. The samples from across Newfoundland were recorded as present/absent data and mapped (Fig. B1).

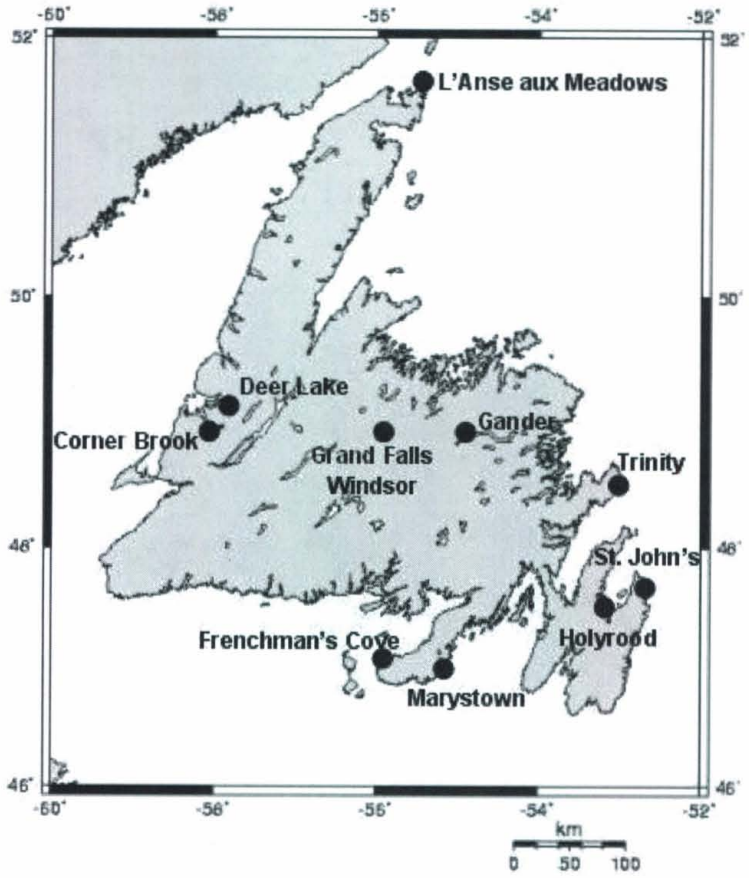

FIGURE B1. Location of sites sampled for NL provincial survey. Areas with damage typical of *B. leucopterus hirtus* were found at all sample locations. Specimens were collected from all sites except L'Anse aux Meadows. Map created by Online Map Creation (http://www.aquarius.geomar.de/omc/).

Results

Blissus leucopterus hirtus is widely distributed across Newfoundland (Fig. A1). Of the ten locations sampled, nine had *B. leucopterus hirtus* present. At L'Anse aux Meadows at the Northern tip of the island, no chinch bugs were found but several areas of damage

resembling that resulting from chinch bug infestations were observed. However it could not be verified that this damage was directly related to *B. leucopterus hirtus*.

Discussion

The results from the Newfoundland survey support anecdotal accounts that *B. leucopterus hirtus* is widely distributed across the island. The one site where specimens of *B. leucopterus hirtus* were not collected was L'Anse aux Meadows at the northern tip of Newfoundland, close to the Labrador border which is the most northerly Island location and has the most extreme climate of the areas sampled. However, this sampling was conducted in September 2005 when most individuals are in later instars and more highly aggregated (Mailloux and Streu 1981). Sampling was difficult on the Northern Peninsula due to the very cool temperatures in this area at the sampling time (approximately 5-10°C), which may have caused these insects to initiate overwintering behaviour earlier than at the other sites. When *B. leucopterus hirtus* prepare for winter they abandon summer habitats for more sheltered and hidden areas (Leonard 1966, Vittum et al. 1999, Potter 1998) and thus may not have been associated with the damaged turf patches. Despite the lack of specimens from L'Anse aux Meadows, several areas of brown turf resembling damage by *B. leucopterus hirtus* were observed. Given its wide spread distribution, it is probable that *B. leucopterus hirtus* inhabits that area, but this will need to be confirmed by further sampling.

Literature Cited

Brown, W. J. 1949. Collected Specimen. 1 female, St. John's, 16.VII. Canadian National Collection, Ottawa, Ontario, Canada.

Leonard, D. E. 1966. Biosystematics of the *leucopterus* complex of the genus *Blissus* (Hemiptera: Lygaeidae). Bulletin - Connecticut Agricultural Experiment Station 677:1-47.

Mailloux, G., and H. T. Streu. 1981. Population biology of the hairy chinch bug (*B. leucopterus hirtus*, Montandon: Hemiptera: Lygaeidae). Annals of the Entomological Society of Quebec 26:51-90.

Potter, D. A. 1998. Destructive turfgrass insects: biology, diagnosis and control. Ann Arbor Press, Chelsea, Michigan, USA.

Vittum, P., M. Villani, and H. Tashiro, 1999. Turfgrass insects of the United States and Canada, 2nd ed. Cornell University Press, New York, USA.

Appendix C: A Comparison of Sampling Methods for *Blissus leucopterus hirtus* Montandon (Hemiptera: Blissidae)

Introduction

Blissus leucopterus hirtus is a widespread turfgrass and crop pest in north eastern North America (Vittum et al. 1999). Its current Canadian range extends from Ontario eastward to Newfoundland. Within the United States, the range extends as far west as Eastern Minnesota and south into North Virginia. The insect attacks plants using piercing and sucking mouthparts to extract moisture. Damage is characterized by yellowish brown patches on lawns and turf (Fig. C1).

Previous studies have a used variety of sampling methods to assess population numbers (Wellwood et al. 2002, Majeau et al. 2000, Mailloux and Streu 1981). There was a need in this study to determine the most appropriate sampling method for various applications. While commercial lawn care companies require quick, on-site methods capable of detecting threshold levels of *B. leucopterus hirtus* and determining if treatment is necessary, scientific research demands replicable methods for accurate, reliable insect counts and insight into population characteristics.

FIGURE C1. Typical lawn damage caused by *B. leucopterus hirtus* (Charbonneau & Sears 2000).

Sampling methods evaluated included:

1. Vacuum: suction of HCBs into a collection cup using vacuum system.
2. Floatation: netting of floating HCBs from an in-situ column of water.
3. Berlese Funnel: exposure to heat of removed sod placed in funnel.
4. Quadrat: visual count in-situ within a small defined area.

The objectives of this study were to compare these four sampling methods for the HCB in lawns, in order to assess

1. Effectiveness, as measured by the number of *B. leucopterus hirtus* individuals captured from a known population.

2. Efficiency, as evaluated according to criteria pertinent to either scientific investigation or commercial application.

Materials and Methods

Sixteen wooden boxes, each measuring 30 cm by 30 cm by 15 cm, were lined with Kentucky bluegrass sod free of *B. leucopterus hirtus*. The boxes were arranged into four blocks of four; each block represented one replicate trial for four methods (Fig. C2a). Fifty individuals of *B. leucopterus hirtus* (5th instars and adults) were released into the centre of each box and the boxes covered with nylon fabric. The insects were allowed to acclimatize for 24 hours. All samples were of identical area, and taken in the centre of each box. Following protocol from previous work, floatation sampling was conducted for 10 minutes and quadrat sampling was 2 minutes (Wellwood et al. 2002, Fig. C2b). Lacking previous examples, 30 seconds was determined to be an adequate time for vacuum sampling (Fig. C2c). Sections of sod removed for the Berlese method were allowed to remain under heat for 24 hours. Samples were filtered where appropriate and the number of *B. leucopterus hirtus* in each sample counted under a stereomicroscope. Various efficiency criteria were rated for each sampling method.

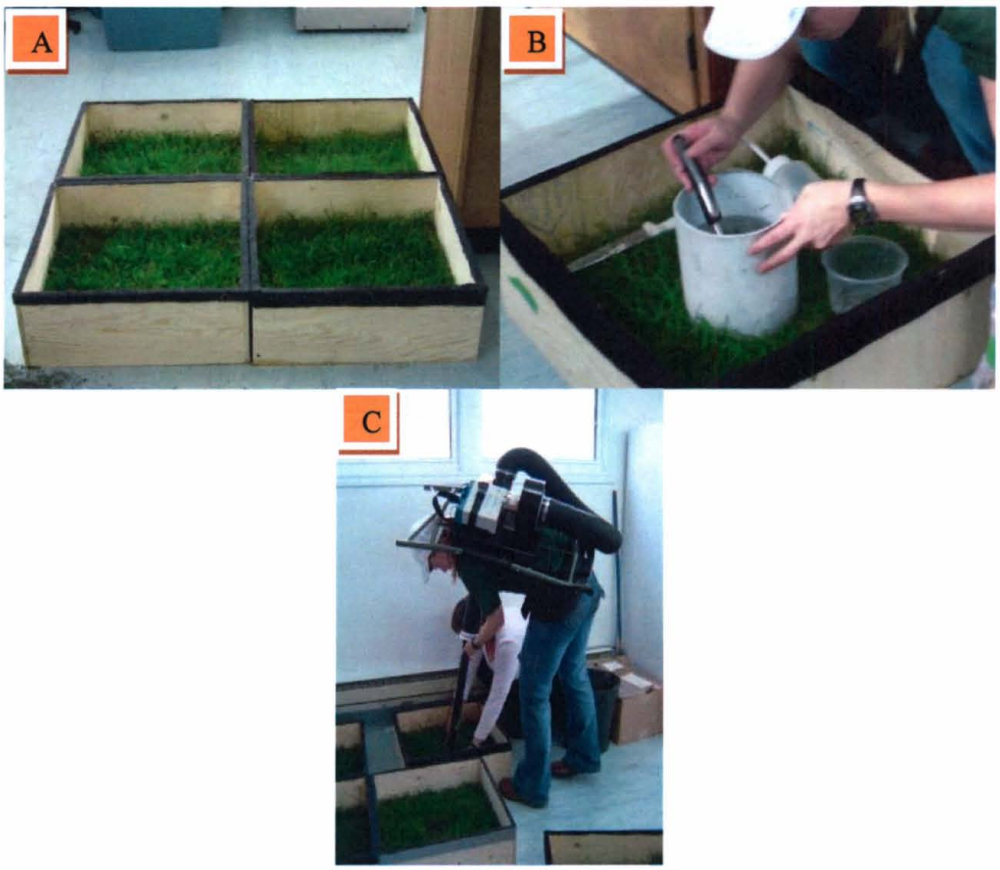

FIGURE C2. Experimental set up and methodology A) Block of four boxes, representing one replicate trial. B) Sampling by floatation. C) Sampling by vacuum system.

Results

Assuming homogeneous distribution and no edge effects, the theoretical recapture rate, based on sampling area, is 10 *B. leucopterus hirtus* per box. A one way ANOVA shows the sampling methods to be statistically different with respect to insect recapture rate ($p=0.002$). Further comparison using a Tukey test shows that, at alpha=0.05, vacuum sampling is significantly different from the quadrat method, and floatation method is significantly different from Berlese funnel and quadrat methods (Fig. B3). An evaluation

of various efficiency criteria for each sampling method showed varying advantages and disadvantages of each method (Table B1).

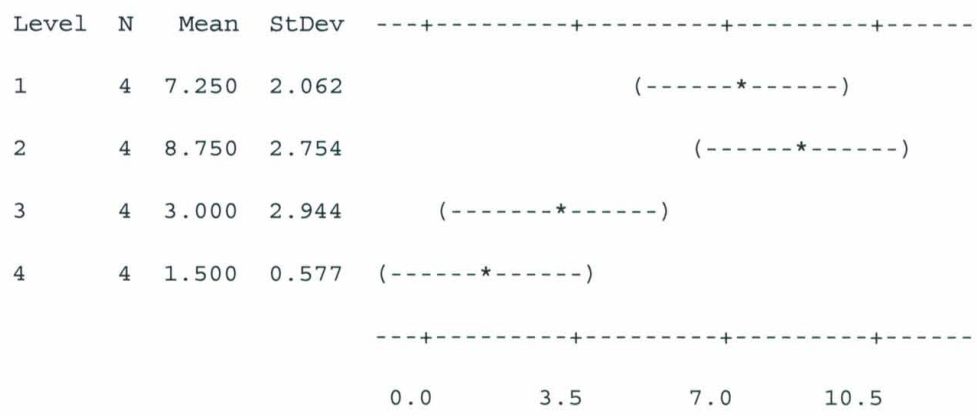

FIGURE C3. Individual 95% CIs for mean based on pooled StDev (MiniTab). Pooled StDev =2.282. Levels: 1=Vacuum, 2=Floatation, 3=Berlese funnel, 4=Quadrat.

TABLE C1. An evaluation of various efficiency criteria for each sampling method.

	Vacuum	**Floatation**	**Berlese**	**Quadrat**
Cost of Equipment	High	Low	Med	Low
User Training	Med	Low	Med	Med
Collection Time	Low	High	Low	Med
Damage to Lawn	Low	Med	High	Low
Sample Process Time	Med	Med-High	High	N/A

Discussion

The floatation method and the vacuum sampling method are comparable in terms of their ability to extract *B. leucopterus hirtus* from the test plots. While the floatation method has traditionally been used for scientific collection of this insect, the vacuum may be considered as a viable option. Although initially high in cost, it reduces both collection and sample process time. The quadrat sampling method offers a low cost, fast method for commercial purposes. However, it is not as effective as other methods in estimating *B. leucopterus hirtus* density. If population numbers are known to be high, it may be an efficient means of conducting threshold counts. The Berlese funnel method was inconsistent and required a high turnover time. It may be more appropriate for thatch oriented sampling. In terms of lawn sampling, it was considered suitable for neither scientific nor commercial applications.

Literature Cited

Charbonneau, P., and M.K. Sears. 2000. Fact sheet: Hairy chinch bugs in lawns. Ontario Ministry of Agriculture and Food. Queen's Printer for Ontario, Canada.

Mailloux G., and H. T. Streu. 1981. Population biology of the hairy chinch bug (*Blissus leucopterus hirtus*, Montandon: Hemiptera: Lygaeidae). Annals of the Entomological Society of Quebec 26:51-90.

Majeau, G., J. Brodeur and Y. Carrière. 2000. Sequential sampling plans for the hairy chinch bug (Hemiptera: Lygaeidae). Journal of Economic Entomology 93:834-839.

Vittum, P., M. Villani, and H. Tashiro. 1999. Turfgrass insects of the United States and Canada, 2nd ed. Cornell University Press, Ithaca, New York, USA.

Wellwood, A., G. Nickerson, and J. Wetmore. 2002. Hairy chinch bug survey, demonstration and monitoring in New Brunswick, 2002. New Brunswick Department of Agriculture, Fisheries and Aquaculture and New Brunswick Horticultural Trades Association, Fredericton, New Brunswick, Canada.

Appendix D. Assessment of Error in Measuring Mensural Characteristics of *B. leucopterus hirtus* Montandon (Hemiptera: Blissidae).

Based on a test of repeated measures, measurement error was greatest in measurements of the 'eye to eye' width and the 'left antenna' length, which are two of the shortest characters and, therefore, most susceptible to micrometer sighting error (Table D1).

TABLE D1. Variation in repeated measurements on the same individual (s^2 within) and on measurements on different individuals (s^2 among). Values taken from paramters within ANOVA model comparing measuresments among individuals using repeated measures on identical individuals as replicates. The % Error within indicates the percentage of error that can be atributed to differences in measurements on the same individual, and % Error among indicated the percentage of error attributed to differences among individuals.

Character	s^2 within	s^2 among	% Error within	% Error among
Eye to Eye	0.001	0.010	11.538	88.462
L Antenna	0.001	0.003	27.582	72.418
R Antenna	0.001	0.019	5.562	94.438
Thorax	0.002	0.185	0.893	99.107
L Corium	0.004	0.562	0.754	99.246
R Corium	0.005	0.570	0.853	99.147
L Wing	0.004	5.969	0.064	99.936
R Wing	0.003	5.972	0.052	99.948
Total Length	0.028	0.355	7.361	92.639

In all characters measured during this study, the variation between individuals was far greater than the variation between repeated measurements on the same individual. The least amount of error for repeated measurements was found in both the left and right wing, while the greatest measurement variation within the same individual occurred in the traits of left antenna and between the outer extremities of the eyes. There are multiple explanations for these results including the reality that large, two dimensional features such as wings are more easily positioned and landmarked than articulated antennae and spherical eyes. Additionally, while wings are fairly large features relative to total body size, eyes and antennae are much smaller. An identical magnification was used for all parts of the body, reducing the relative precision of the eye to eye and antennal measurements (i.e. a difference of one ocular unit in measurements represents a larger proportion of the total feature length in eyes compared to wings).

Appendix E: Correlation of Mensural Characteristics in *B. leucopterus hirtus* Montandon (Hemiptera: Blissidae).

The matrix below compares each character measure to every other character measure and provides a correlative value (Fig. E1). Following the matrix a table of correlation values between each set of measurements is found (Table E1).

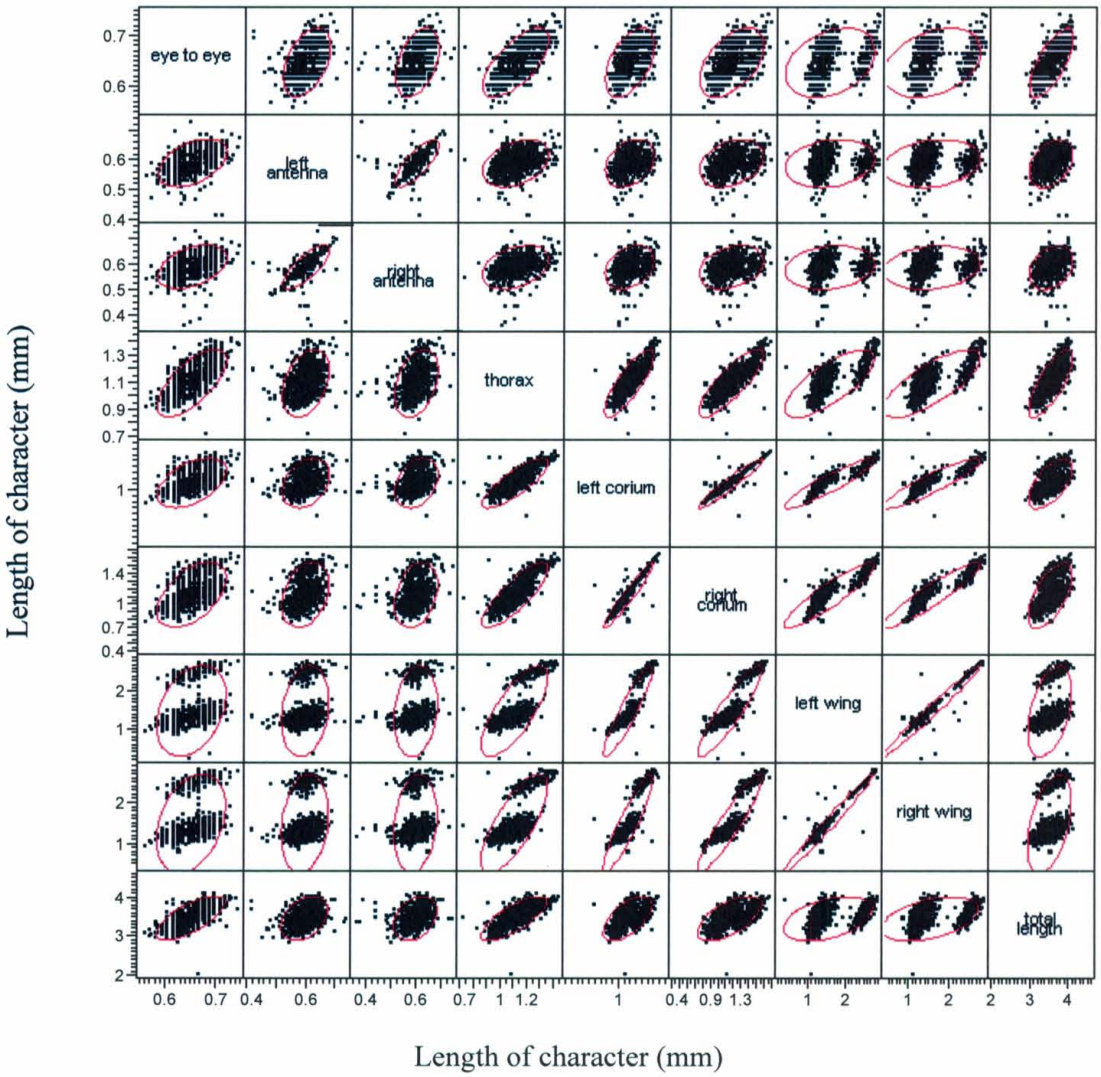

FIGURE E1. Scatterplot matrix of nine measured traits in 12 populations of *B. leucopterus hirtus*. Sex and wing forms are combined (n=1351). Boxes showing two distinct groups highlight the dimorphic nature of traits such as corium length and wing length. Correlative values are also given (Table 3.3). A strong correlation between symmetrically equivalent characters suggests isometry. Red ellipses represent 95% confidence interval.

TABLE E1. Correlations between nine measured characteristics in 1351 specimens of *B. leucopterus hirtus* combined from 12 populations across Quebec and Atlantic Canada.

	eye to eye	left antenna	right antenna	thorax	left corium	right corium	left wing	right wing	total length
eye to eye	1	0.4543	0.4240	0.6837	0.5003	0.4980	0.3240	0.3257	0.7312
left antenna	0.4543	1	0.7756	0.3647	0.3097	0.2989	0.1946	0.1933	0.3751
right antenna	0.4240	0.7756	1	0.3536	0.2946	0.2838	0.1770	0.1761	0.3341
thorax	0.6837	0.3647	0.3536	1	0.8350	0.8357	0.7488	0.7513	0.7812
left corium	0.5003	0.3097	0.2946	0.8350	1	0.9621	0.9143	0.9081	0.6211
right corium	0.498	0.2989	0.2838	0.8357	0.9621	1	0.9061	0.9266	0.6313
left wing	0.3240	0.1946	0.1770	0.7488	0.9143	0.9061	1	0.9864	0.4508
right wing	0.3257	0.1933	0.1761	0.7513	0.9081	0.9266	0.9864	1	0.4616
total length	0.7312	0.3751	0.3341	0.7812	0.6211	0.6313	0.4508	0.4616	1

Appendix F: Abnormalities in Collected Specimens of *B. leucopterus hirtus* Montandon (Hemiptera: Blissidae).

The level of abnormality in the measured specimens ranged from 5% in NB3 to 29% in NS2. These are considered conservative estimates since all deformities may not have been noted, as they were only recorded through casual inspection. Despite these deformities, there was a strong correlation between features on the left and right sides of the body, indicating that growth was primarily isometric for symmetrically opposing characteristics (Fig. D1). Several characters were specifically examined, and there was, for example, no significant difference in the frequency of wing asymmetry among sites for either corium ($p=0.1499$) or wing measurements ($p=0.1203$). Examples of abnormalities are shown (Fig. F1).

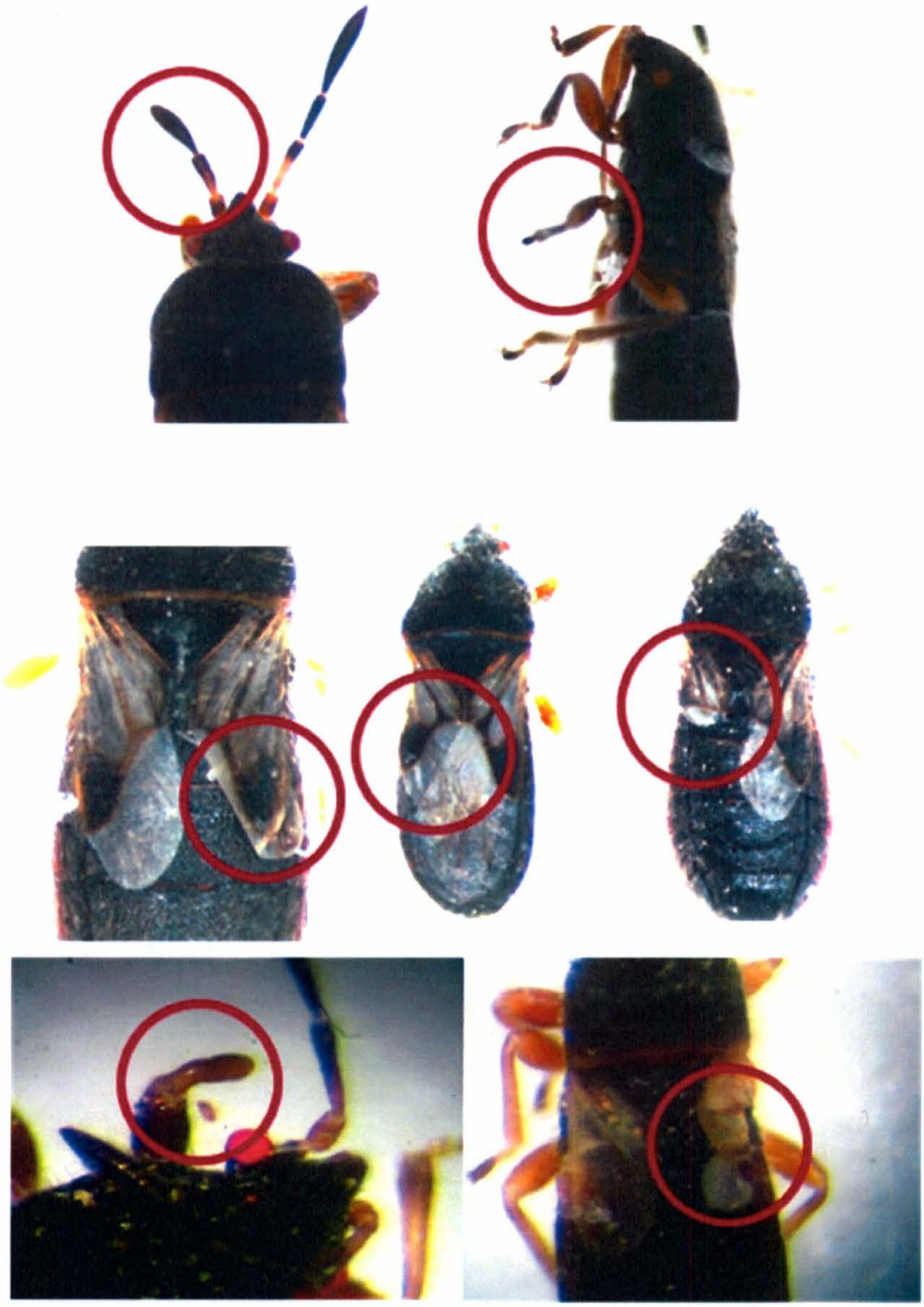

FIGURE F1. Examples of aberrations observed.

Existing literature on the rate of morphological aberrations in Hemiptera is limited. However, evidence of 'compensatory hyper-regeneration' has been suggested as an explanation for some antennal abnormalities in Hemiptera (Wolsky 1957) after experimental replication of this process was conducted in *Euschistus variolarius* Palisot (Hemiptera: Pentatomidae) and *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae). When portions of the antennae were lost during early nymphal stages, later forms exhibited exaggerated length in the remaining segments, although the original length was rarely obtained. Wolsky's proposed explanation for this process is the enlargement of cells through endomitosis, a widespread phenomenon in Hemiptera (Wolsky 1957).

Considering the large number of aberrations observed in these populations of *B. leucopterus hirtus*, particularly in the antennae, the importance (or lack thereof) of these appendages in the insect's survival is of further interest. The importance of antennal function is not completely understood in *B. leucopterus hirtus*, but it is clear that these appendages are used in courtship, and vibrational movement of the antennae in females when in contact with males may indicate willingness to mate (Leonard 1966). It is unclear what the implication of such aberrations is to fitness. A definitive explanation for the presence of aberrations or variability in frequency among populations of *Blissus leucopterus hirtus* is also not clear. However, this area deserves additional attention based on the conservative estimate of 5-29% frequency spectrum in anomalies observed. Despite some individuals displaying obvious aberrations in antennae, wings and other appendages, most adults displayed relative symmetry in left and right characteristics.

Differences between wing length were usually very small (<0.25 mm) and the overall correlation between left and right was very high.

Literature Cited

Leonard, D. E. 1966. Biosystematics of the *leucopterus* complex of the genus *Blissus* (Hemiptera: Lygaeidae). Bulletin - Connecticut Agricultural Experiment Station 677:1-47.

Wolsky, A. 1957. 'Compensatory hyper-regeneration' in the antennae of Hemiptera. Nature 180:1144-1145.

Appendix G: Multivariate Analysis to Discriminate Sex, Wing form and Population of Origin

The data were subjected to multivariate analysis to determine whether the information collected is sufficient to discriminate among populations at both the local and regional level. Canonical variate (multiple discriminate) analysis was used to test predictive power. The data were first screened according to a multivariate data checklist provided in Tabachnick and Fidell (2000). This involved 1) inspecting data using descriptive statistics to check for accuracy of input, 2) evaluating the amount and distribution of the data. In this case, missing data were substituted with the mean value of existing values for that character 3) checking pairwise plots for non-linearity and heteroscedasticity, 4) identifying and dealing with non-normal variables, and finally 5) identifying and dealing with multivariate outliers

A total of 1351 adult *B. leucopterus hirtus* were initially considered for this analysis. Because of the close correlation between symmetrically opposing sides of the characteristics (Appendix D), only the measurements taken from the left side were used in further comparisons to avoid the effects of multicollinearity. There was also a high correlation between corium length and wing length on both the left (r^2=0.9143) and right (r^2=0.9266) sides, so corium length was eliminated from further analysis. Brachypterous and macropterous forms were considered separately because of their distinct size differences. Data fill resulted in estimation of 5.4% of the data for long winged individuals and 5.1% of the data for short winged individuals. Discriminant analysis was

able to predict sex among the individuals based on eye to eye width, left antenna width, thorax width, wing length and total length with 82.91% correct matches in macropterous individuals (Fig. G1) and 89.9% correct matches in brachypterous individuals (Fig. G2).

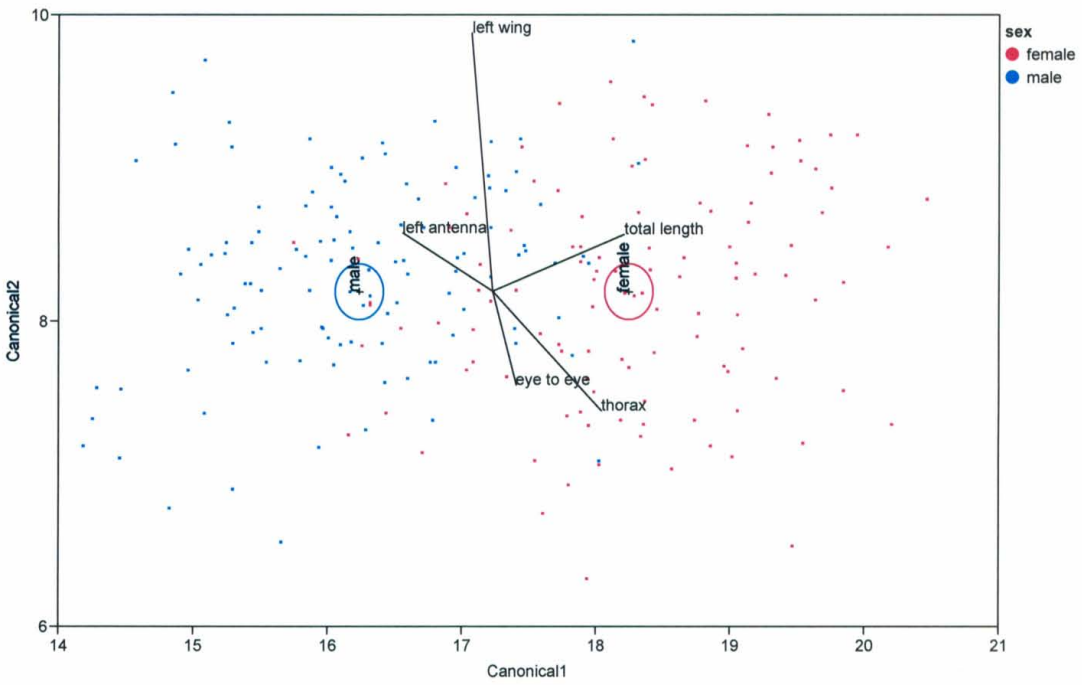

FIGURE G1. Canonical plot of macropterous *B. leucopterus hirtus* showing discrimination by sex based on five mensural characteristics. There was 82.91% correct classification. The two crosses marked with M and F show the mean value, with the circle surrounding them showing the 95% confidence interval for each sex. Each dot represents one bug, and the dots have been made partially transparent to reduce visual crowding. The ray plot shows the vectors associated with each characteristic used in discrimination.

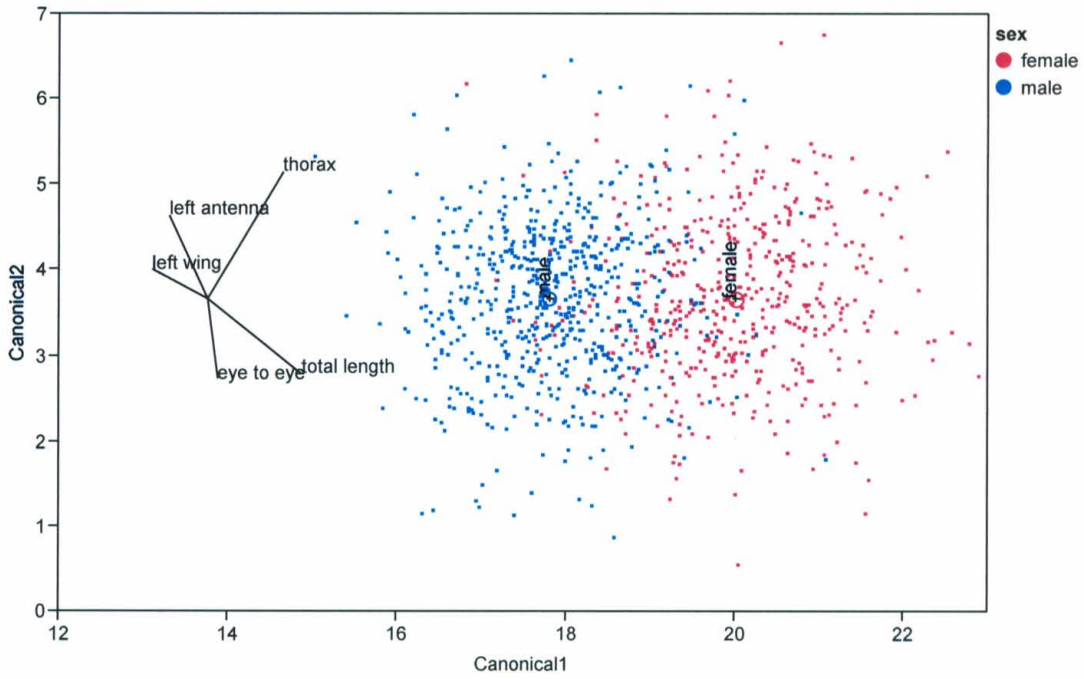

FIGURE G2. Canonical plot of brachypterous *B. leucopterus hirtus* showing discrimination by sex based on five mensural characteristics. There was 89.9% correct classification. The two small crosses marked with male and female show the mean value, with the circle surrounding them showing the 95% confidence interval for each sex; in this case, the circle is very small. Each dot represents one bug, and the dots have been made partially transparent to reduce visual crowding. The ray plot shows the vectors associated with each character used in discrimination and is shown to the left of the scatterplot to facilitate reading.

The characters of eye to eye, left antenna, thorax and total length were also used to predict wing form. There was a high correct classification rate for both females (95.63%) (Fig. G3) and males (96.32%) (Fig. G4). Discriminate analysis using sex, eye to eye, left antenna, thorax, left wing and total length could not predict site with accuracy, resulting in only 18.80% correct classification (Fig. G5). Results were improved for prediction of province of origin, with a 44.04% classification rate (Fig G6).

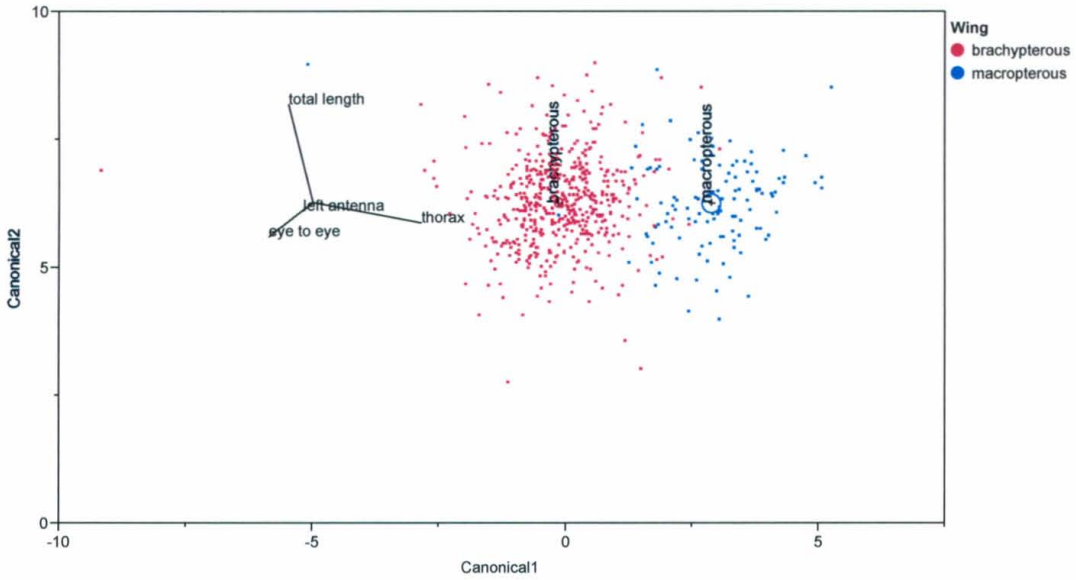

FIGURE G3. Canonical plot of female *B. leucopterus hirtus* showing discrimination by wing form based on four mensural characteristics. There was 95.63 % correct classification. The two small crosses marked with brachypterous and macropterous show the mean value, with the circle surrounding them showing the 95% confidence interval for each sex; in this case, the circle is very small. Each dot represents one bug, and the dots have been made partially transparent to reduce visual crowding. The ray plot shows the vectors associated with each character used in discrimination and is shown to the left of the scatterplot to facilitate reading.

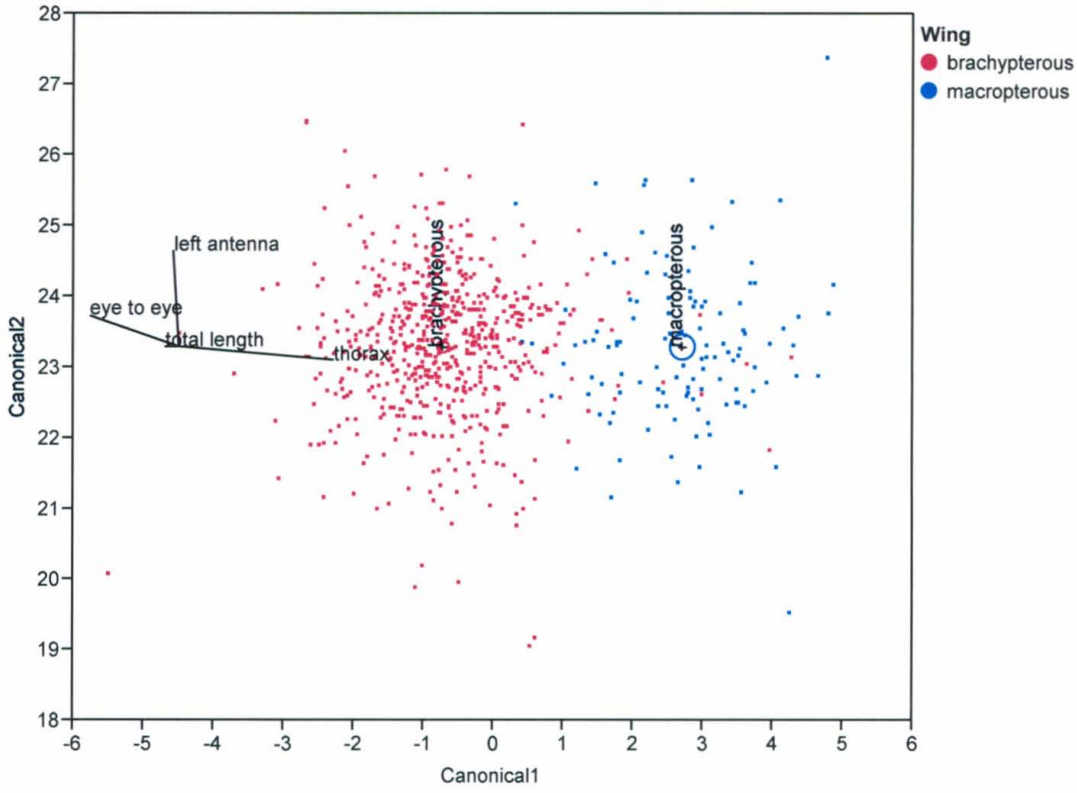

FIGURE G4. Canonical plot of male *B. leucopterus hirtus* showing discrimination by wing form based on four mensural characteristics. There was 96.32 % correct classification. The two small crosses marked with brachypterous and macropterous show the mean value, with the circle surrounding them showing the 95% confidence interval for each sex; in this case, the circle is very small. Each dot represents one bug, and the dots have been made partially transparent to reduce visual crowding. The ray plot shows the vectors associated with each characteristic used in discrimination and is shown to the left of the scatterplot to facilitate reading.

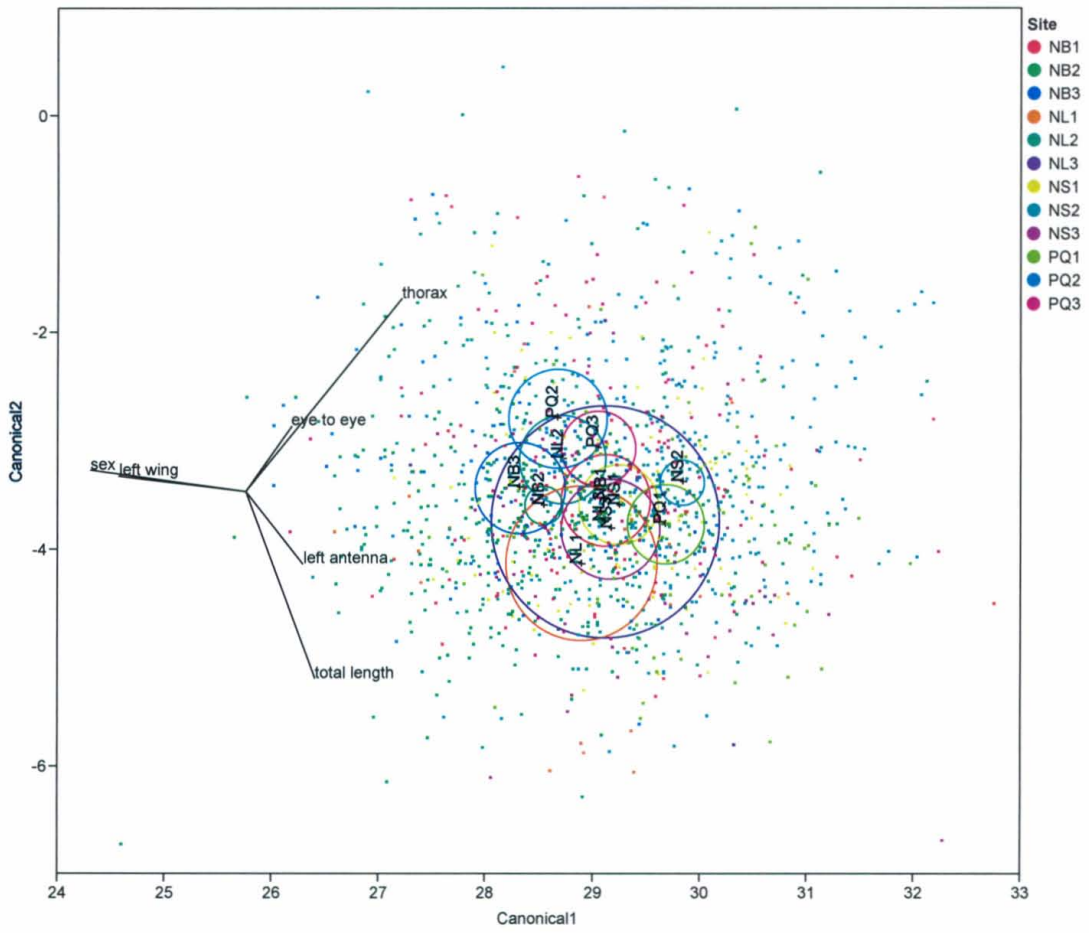

FIGURE G5. Canonical plot for *B. leucopterus hirtus* showing discrimination by site based on six mensural characteristics. The small crosses marked with site names show the mean value, with the circle surrounding them showing the 95% confidence interval for each site mean. Each dot represents one bug, and the dots have been made partially transparent to reduce visual crowding. The ray plot shows the vectors associated with each characteristic used in discrimination and is shown to the left of the scatterplot to facilitate reading.

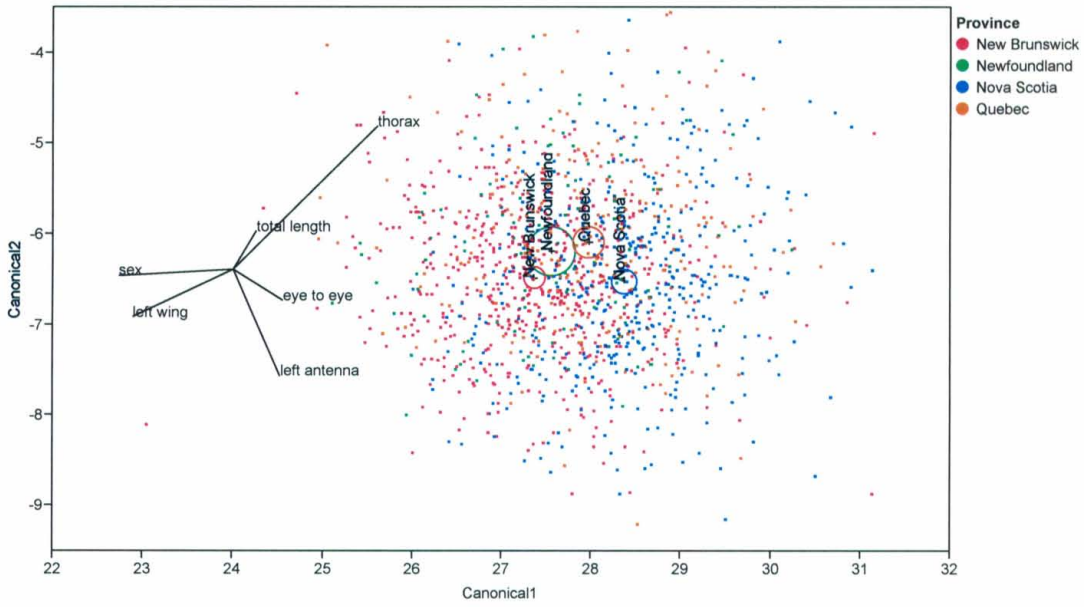

FIGURE G6. Canonical plot for *B. leucopterus hirtus* showing discrimination by province based on six mensural characteristics. The small crosses marked with province names show the mean value, with the circle surrounding them showing the 95% confidence interval for each site mean. Each dot represents one bug, and the dots have been made partially transparent because of crowding. The ray plot shows the vectors associated with each characteristic used in discrimination and is shown to the left of the scatterplot to facilitate reading.

Based on the significant difference in length/width of characters for both sexes and wing form for most characters, discriminate analysis was effective in separating both males and females and macropterous and brachypterous wing forms. However, the data collected did not classify individuals into their twelve sites of origin. Regional sorting was slightly improved, but despite the means of most characters being significantly different for at least one region for all characters (Appendix F), there was sufficient individual variation within the populations to inhibit complete differentiation based on morphometrics.

Literature Cited

Tabachnick, B. G., and L. S. Fidell. 2000. Using multivariate statistics, 4th ed. Allyn and Bacon, Boston, Massachusetts, USA.